THE AMELIORATING AND ANTIOXIDANT STUDY OF AQUEOUS EXTRACT OF *Citrullus lanatus* (Thunb.) PULP USING SCOPOLAMINE INDUCED LEARNING AND MEMORY IMPAIRMENT IN SWISS ALBINO MICE

A Dissertation submitted to

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MASTER OF PHARMACY
IN
BRANCH – IV → PHARMACOLOGY

Submitted by

DIVYA N
261625004

Under the Guidance of
Dr. P. AMUDHA, M.Pharm., Ph.D
Professor
Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY,
C.L.BAID METHA COLLEGE OF PHARMACY,
JYOTHI NAGAR, RAJIV GANDHI SALAI,
THORAPAKKAM, CHENNAI – 600 097.

MAY 2018

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

ISO 9001 : 2008
Reg. No : RQ91/3038

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

Dr. P. Amudha, M.Pharm., Ph.D,

Professor, Department of Pharmacology

CERTIFICATE

This is to certify that the project entitled "**The Ameliorating and Antioxidant Study of Aqueous Extract of *Citrullus lanatus* (Thunb.) Pulp using Scopolamine Induced Learning and Memory Impairment in Swiss Albino Mice"** was submitted by **Divya N (261625004)** in partial fulfillment for the award of the degree of **Master of Pharmacy**. It was carried out at C.L. Baid Metha College of Pharmacy, Chennai – 600097 under my guidance and supervision in the Department OF Pharmacology during the academic year 2017 – 2018.

Date: **Dr. P. Amudha, M.Pharm., Ph.D,**

Place: Chennai

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

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L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

Dr. P. Muralidharan, M.Pharm., Ph.D,

Professor & HOD, Department of Pharmacology

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Date: **Dr. P. Muralidharan, M.Pharm., Ph.D,**

Place: Chennai

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

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Principal

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Date: **Dr. Grace Rathnam, M.Pharm., Ph.D**

Place: Chennai

DECLARATION

The thesis entitled "**The Ameliorating and Antioxidant Study of Aqueous Extract of *Citrullus lanatus* (Thunb.) Pulp using Scopolamine Induced Learning and Memory Impairment in Swiss Albino Mice"** was carried out in Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai – 600097 during the academic year 2017 – 2018. The work embodied in this thesis is original and is not submitted in part or full for any other degree of this or any other university.

Date: Divya N

Place: Chennai Reg no. 261625004

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Date: Divya N

Place: Chennai Reg no. 261625004

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List of Abbreviations

%	Percentage
5-HT	5- Hydroxytryptamine
ACh	Acetylcholine
AchE	Acetylcholinesterase
AchEI	Acetylcholinesterase Inhibitor
AECL	Aqueous Extract of *Citrullus Lanatus*
AD	Alzheimers Disease
ANOVA	Analysis of variance
APO	Apolipoprotein
APP	Amyloid Precursor Protein
BACE	Beta Secretase
BDNF	Brain-Derived Neurotrophic Factor
CA	Cornu Ammonis
CAT	Catalase
CCl_4	Carbon tetrachloride
cm	Centimeter
CO_2	Carbon di oxide
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CT	computerized tomography
D	Dopaminergic
DNA	Deoxyribonucleic Acid
DWI	Diffusion Weighed Imaging
EDTA	Ethylene Diamine Tetra Acetic acid
EEG	Electroencephalogram
EL	Escape Latency
EPM	Elevated Plus Maze
EPO	Erythropoietin
GABA	Gamma-aminobutyric acid
GPx	Glutathione Peroxidation
GSK	Glycogen Synthase Kinase
HMG COA	3-hydroxy-3-methyl-glutaryl-coenzyme A
H_2O_2	Hydrogen Peroxide
i.p	Intraperitonial
IAEC	Institutional Animal Ethics Committee
IVIG	Intravenous Immunoglobulin
Kg	Kilogram
L	Litre
LPO	Lipid Peroxidation
M	Mole
MDA	Malondialdehyde
Mg	Milligram
Min	Minutes
MRI	Magnetic Resonance Imaging
MWM	Morris Water Maze
NADH	Nicotinamide adenine dinucleotide
$NaNO_2$	Sodium Nitrite

NBT	Nitroblue tetrazolium
Nm	Nanometer
NMDA	N-Methyl-D-aspartic acid
NRI	norepinephrine reuptake inhibitor
O_2	Oxygen
PMS	Phenazine methosulphate
PA	Passive Avoidance
PaO_2	partial pressure of oxygen
Ph	potential hydrogen
PO_4	Phosphate
PTSD	Posttraumatic stress disorder
RA	Retrograde Amnesia
RL	Retention Latency
ROS	Reactive Oxygen Species
rpm	Rotations per minute
sec	Seconds
SEM	Standard Error of the Mean
SOD	Suproxide Dismutase
SDS	Sodium dodecy Sulphate
SSRI	Selective serotonin reuptake inhibitor,
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
TIA	Transient Ischemic Attack
TL	Transfer Latency
WHO	World health Organisation
α	Alpha
B	Beta

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LIST OF CHEMICALS

S.No	Chemical	Manufacturer
1	1,1,3,3 - tetramethoxy propane	Sigma Aldrich
2	5,5-Dithio-bis-2-nitro benzic acid (DTNB) Ellman's reagent	Sisco Research Laboratories pvt. Ltd, Mumbai
3	Acetic acid	S.D. FineChem Ltd, Mumbai
4	Acetylthiocholine iodide	S.D. FineChem Ltd, Mumbai
5	Anhydrous Sodium Phosphate Monobasic	Pari Chemicals
6	Chloroform	LobaChemie Pvt. Ltd, Mumbai
7	Distilled water	Andavar Distilled Water Company, Chennai
8	Ethanol	Alpha Chemika
9	Ethylene diamine tetra acetic acid (EDTA) Disodium Salt	Chemspure, Chennai 12
10	Formalin	Paxy speciality chemicals, Chennai
11	Glacial Acetic Acid	Alpha Chemika
12	Glutathione Solution	SRL chemicals, sisco lab, Maharastra
13	Hydrogen peroxide	Chemspure, Chennai
14	NADH Nicotinamide adenine dinucleotide	Sigma Aldrich
15	n-Butanol	SRL chemicals, sisco lab, Maharastra
16	Nitroblue tetrazolium	SRL chemicals, sisco lab, Maharastra
17	Phenazine methosulphate	Chemsworth
18	Piracetam	Dr Reddy's Laboratories Ltd.
19	Potassium chloride	Chemspure, Chennai
20	Potassium Dichromate	SRL chemicals, sisco lab, Maharastra
21	Pyridine	SRL chemicals, sisco lab, Maharastra
22	Scopolamine	Vitas Healthcare Ltd
23	Sodium Azide	SRL chemicals, sisco lab, Maharastra
24	Sodium Dodecy Sulphate	SRL chemicals, sisco lab, Maharastra
25	Sodium Phosphate	SRL chemicals, sisco lab, Maharastra
26	Sodium Phosphate Dibasic	LobaChemie Pvt. Ltd, Mumbai
27	Sodium Pyrophosphate	SRL chemicals, sisco lab, Maharastra
28	Thiobarbuturic Acid	Sigma Aldrich
29	Trichloroacetic Acid	SRL chemicals, sisco lab, Maharastra

1. INTRODUCTION

Nature is the abundant source of medicinal plants. The use of medicinal plants is from ancient times onward. Till today majority of people rely on such traditional remedies. Many of compounds used for the production of modern medicines were also derived from the herbs in the surroundings.

Because of their ability to synthesize a wide variety of chemical compounds that can be used to perform important biological functions, their phytochemicals have been processed for beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total.[1]

The term "herbs" refers to plants or parts of them, including grasses, flowers, berries, seeds, leaves, nuts, stems, stalks and roots, which are used for their therapeutic and health- enhancing properties. Generations of skilled herbal practitioners, researchers and scholars have refined and tested the vast science of herbology, producing thousands of plant-based remedies that are safe and effective.

An estimated eighty percent (80%) of the world's population employs herbs as primary medicines

Ayurveda, Siddha and Unani systems of medicine provide good base for scientific exploration of medicinally important molecules from nature.

India is rightly called the botanical garden of the world and perhaps the largest producer of medicinal herbs. India recognizes over 3000 plants for their medicinal value. It is estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine i.e. 75% of the medicinal needs of the world countries. Medicinal herbs are used in one form or another, under indigenous systems of medicine like Ayurveda, Siddha & Unani.

India holds its history in herbal remedy and found to be so popular that the government of India has created a separate department 'AYUSH' under the Ministry of Health & Family Welfare. In 2000, the National Medicinal plants Board was established by Indian government in order to deal with the herbal medicinal system.

Traditional therapy is supported by evidence on safety and effectiveness. Usually, these evidences were found on sources such as pharmacopoeias, traditional scriptures, and/ or clinical experience registered over hundreds of years. At present, an increasing number of scientific studies support the use of herbal therapy. The advantages of traditional medicine include its diversity and flexibility, availability and affordability. It is of comparatively low cost and merely requires relatively low level of technological input. However, there is a need for an increase in research to improve the evidence base as regards to efficacy.[2]

Plant Medicines, Safer And Time-Tested. Plant medicines are far and away safer, gentler and better for human health than synthetic drugs. This is so because human beings have co-evolved with plants over the past few million years.[3]

2. LITERATURE REVIEW

2.1 DEFINITION

Amnesia is the main feature is loss of memory, usually of important recent events, that is not due to organic mental disorder, and is too great to be explained by ordinary forgetfulness or fatigue. The amnesia is usually centred on traumatic events, such as accidents or unexpected bereavements, and is usually partial and selective. Complete and generalized amnesia is rare, and is usually part of a fugue. If this is the case, the disorder should be classified as such. The diagnosis should not be made in the presence of organic brain disorders, intoxication, or excessive fatigue. Scopolamine is a muscarinic receptor antagonist with profound amnesic effects in a variety of learning paradigms and a useful experimental pharmacological model to investigate the pathophysiology of the cognitive deficit in AD.[4]

Learning is defined as the acquisition of any new information about the events occurring in surroundings and subsequent retention and retrieval of this information is referred to as memory[5]. Diminished cholinergic firing in brain[6], rise in oxidative stress[7], neuroinflammatory reactions[8], hypercholesterolemia[9] have been exhibited to play an etiological role in memory deficits. Most of presently used therapeutic interventions provide only symptomatic relief and do not halt the progression of memory deficits; further associated side effects too limit their use. Therefore, there is need for new directions of managing such cognition disorders which will not only provide relief from symptoms but will also stop the progression of memory deficits.

2.2 MEMORY[10]

Memory is the processes by which information is encoded, stored, and retrieved.

There are three main stages in the formation and retrieval of memory:

Encoding or registration (receiving, processing and combining of received information)

- Storage (creation of a permanent record of the encoded information)
- Retrieval, recall or recollection (calling back the stored information in response to some clue for use in a process or activity)
- Encoding allows information that is from the outside world to reach our senses in the forms of chemical and physical stimuli. In this first stage we must change the information so that we may put the memory into the encoding process. Storage is the second memory stage or process. This entails that we maintain information over periods of time. Finally the third process is retrieval. This is the retrieval of information that we have stored. We must locate it and return it to our consciousness. Some retrieval attempts may be effortless due to the type of information.

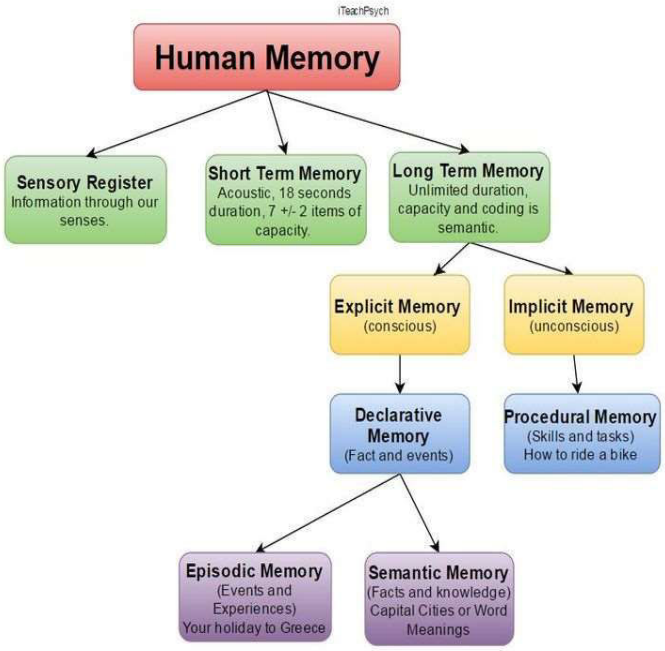


Figure 1: Types of Memory

Sensory Memory

Sensory memory corresponds approximately to the initial 200– 500 milliseconds after an item is perceived. The ability to look at an item, and remember what it looked like with just a second of observation, or memorisation, is an example of sensory memory.

Short term memory

Short-term memory allows recall for a period of several seconds to a minute without rehearsal. Its capacity is also very limited. However, memory capacity can be increased through a process called chunking

Long-term memory

Long-term memory can store much larger quantities of information for potentially unlimited duration (sometimes a whole life span). Its capacity is immeasurably large. Longterm memories, on the other hand, are maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential (for learning new information) to the consolidation of information from short-term to long-term memory, although it does not seem to store information itself. Without the hippocampus, new memories are unable to be stored into long-term memory, as learned from HM after removal of his hippocampus, and there will be a very short attention span. Furthermore, it may be involved

in changing neural connections for a period of three months or more after the initial learning. Several studies have demonstrated that memory depends on getting sufficient sleep between training and test. Additionally, data obtained from neuroimaging studies have shown activation patterns in the sleeping brain which mirror

those recorded during the learning of tasks from the previous day, suggesting that new memories may be solidified through such rehearsal.

2.3 AMNESIA

Amnesia is a condition in which one's memory is lost. The causes of amnesia have traditionally been divided into certain categories. Memory appears to be stored in several parts of the limbic system of the brain, and any condition that interferes with the function of this system can cause amnesia. Functional causes are psychological factors, such as posttraumatic stress or, in psycho analytic terms, defense mechanism

2.3.1 Types Of Amnesia

Anterograde amnesia is the inability to create new memories due to brain damage, while long-term memories from before the event remain intact. The brain damage can be caused by the effects of long-term alcoholism, severe malnutrition, stroke, head trauma, encephalitis, surgery, Wernicke–Korsakoff syndrome, cerebrovascular events, anoxia or other trauma. The two brain regions related with this condition are medial temporal lobe and medial diencephalon. Anterograde amnesia cannot be treated with pharmacological methods due to neuronal loss. However, treatment exists in educating patients to define their daily routines and after several steps they begin to benefit from their procedural memory. Likewise, social and emotional support is critical to improving quality of life for anterograde amnesia sufferers.[11]

Retrograde amnesia is inability to recall memories before onset of amnesia. One may be able to encode new memories after the incident. Retrograde is usually caused by head trauma or brain damage to parts of the brain besides the hippocampus. The hippocampus is responsible for encoding new memory. Episodic memory is more likely to be affected than semantic memory. The damage is usually caused by head trauma, cerebrovascular accident, stroke, tumor, hypoxia, encephalitis, or chronic alcoholism. People suffering from retrograde amnesia are more likely to remember general knowledge rather than specifics. Recent memories are less likely to be recovered, but older memories will be easier to recall due to strengthening over time.[12] Retrograde amnesia is usually temporary and can be treated by exposing them to memories from the loss.[13] Another type of consolidation (process by which memories become stable in the brain) occurs over much longer periods of time/days, weeks, months and years and likely involves transfer

of information from the hippocampus to more permanent storage site in the cortex. The operation of this longer-term consolidation process is seen in the retrograde amnesia of patients with hippocampal damage who can recall memories from childhood relatively normally, but are impaired when recalling experiences that occurred just a few years prior to the time they became amnesic.

Post-traumatic amnesia is generally due to a head injury (example: a fall, a knock on the head). Traumatic amnesia is often transient, but may be permanent or either anterograde, retrograde, or mixed type. The extent of the period covered by the amnesia is related to the degree of injury and may give an indication of the prognosis for recovery of other functions. Mild trauma, such as a car accident that results in no more than mild whiplash, might cause the occupant of a car to have no memory of the moments just before the accident due to a brief interruption in the short/long-term memory transfer mechanism. The sufferer may also lose knowledge of who people are. Having longer periods of amnesia or consciousness after an injury may be an indication that recovery from remaining concussion symptoms will take much longer.[14]

Dissociative amnesia results from a psychological cause as opposed to direct damage to the brain caused by head injury, physical trauma or disease, which is known as organic amnesia. Dissociative amnesia can include:

Repressed memory is the inability to recall information, usually about stressful or traumatic events in persons' lives, such as a violent attack or disaster. The memory is stored in long-term memory, but access to it is impaired because of psychological defense mechanisms. Persons retain the capacity to learn new information and there may be some later partial or complete recovery of memory. Formerly known as "Psychogenic Amnesia".

Dissociative fugue (*formerly* psychogenic fugue) is also known as fugue state. It is caused by psychological trauma and is usually temporary and unresolved, and therefore may return. An individual with dissociative fugue disorder is unaware or confused about his or her identity and will travel in journeys away from familiar surroundings to discover or create new identities. The Merck Manual defines it as "one or more episodes of amnesia in which patients cannot recall some or all of their past and either lose their identity or form a new identity[15]. The episodes, called fugues, result from trauma or stress. Dissociative fugue often manifests as sudden, unexpected, purposeful travel away from home." While popular in fiction, it is extremely rare.

Posthypnotic amnesia occurs when events during hypnosis are forgotten, or where past. memories are unable to be recalled. The failure to remember those events is induced by suggestions made during the hypnosis.[16]

Lacunar amnesia is the loss of memory about one specific event.

Childhood amnesia (also known as infantile amnesia) is the common inability to remember events from one's own childhood. Sigmund Freud notoriously attributed this to sexual repression, while modern scientific approaches generally attribute it to aspects of brain development or developmental psychology, including language development, which may be why people do not easily remember pre-language events. Researchers have found that implicit memories cannot be recalled or described. Remembering how to play the piano is a common example of implicit memory, as is walking, speaking and other everyday activities that would be difficult to focus on if they had to be relearned every time one got up in the morning. Explicit memories, on the other hand, can be recalled and described in words. Remembering the first time meeting a teacher is an example of explicit memories.[17]

Transient global amnesia is a well-described medical and clinical phenomenon. This form of amnesia is distinct in that abnormalities in the hippocampus can sometimes be visualized using a special form of magnetic resonance imaging of the brain known as diffusion-weighted imaging (DWI). Symptoms typically last for less than a day and there is often no clear precipitating factor or any other neurological deficits. The cause of this syndrome is not clear. The hypothesis of the syndrome includes transient reduced blood flow, possible seizure or an atypical type of a migraine. Patients are typically amnestic of events more than a few minutes in the past, though immediate recall is usually preserved.

Source amnesia is the inability to remember where, when or how previously learned information has been acquired, while retaining the factual knowledge.[18]

Korsakoff's syndrome can result from long-term alcoholism or malnutrition. It is caused by brain damage due to a vitamin B_1 deficiency and will be progressive if alcohol intake and nutrition pattern are not modified. Other neurological problems are likely to be present in combination with this type of Amnesia. Korsakoff's syndrome is also known to be connected with confabulation. The person's short-term memory may appear to be normal, but the person may have a difficult time attempting to recall a past story, or with unrelated words, as well as complicated patterns. [19]

Drug-induced amnesia is intentionally caused by injection of an amnestic drug to help a patient forget surgery or medical procedures, particularly those not performed under full anesthesia, or likely to be particularly traumatic. Such drugs are also referred to as "premedicants". Most commonly, a 2-halogenated benzodiazepine such as midazolam or flunitrazepam is the drug of choice, although other strongly amnestic drugs such as propofol or scopolamine may also be used for this application. Memories of

the short time-frame in which the procedure was performed are permanently lost or at least substantially reduced, but once the drug wears off, memory is no longer affected.

Situation-specific amnesia can arise in a variety of circumstances (for example, committing an offence, child sexual abuse) resulting in PTSD. It has been claimed that it involves a narrowing of consciousness with attention focused on central perceptual details and/or that the emotional or traumatic events are processed differently from ordinary memories.

Transient epileptic amnesia is a rare and unrecognized form of temporal lobe epilepsy, which is typically an episodic isolated memory loss. nsIt has been recognized as a treatment-responsive syndrome congenial to anti-epileptic drugs.[20]

2.4 ETIOLOGY

There are three generalized categories in which amnesia could be acquired by a person. The three categories are head trauma (example: head injuries), traumatic events (example: seeing something devastating to the mind), or physical deficiencies (example: atrophy of the hippocampus). The majority of amnesia and related memory issues derive from the first two categories as these are more common and the third could be considered a subcategory of the first.

- Head trauma is a very broad range as it deals with any kind of injury or active action toward the brain which might cause amnesia. Retrograde and anterograde amnesia is more often seen from events like this, an exact example of a cause of the two would be electroshock therapy, which would cause both briefly for the receiving patient.
- Traumatic events are more subjective. What is traumatic is dependent on what the person finds to be traumatic. Regardless, a traumatic event is an event where something so distressing occurs that the mind chooses to forget rather than deal with the stress. A common example of amnesia that is caused by traumatic events is dissociative amnesia, which occurs when the person forgets an event that has deeply disturbed them. An example would be a person forgetting a fatal and graphic car accident involving their loved ones.
- Physical deficiencies are different from head trauma because physical deficiencies lean more toward passive physical issues.
- Electroconvulsive therapy in which seizures are electrically induced in patients for therapeutic effect can have acute effects including both retrograde and anterograde amnesia.
- Alcohol can both cause blackouts and have deleterious effects on memory formation.

- Severe illness
- High fever
- Seizures
- Certain drugs, such as barbiturates or heroin
- General anaesthetics
- Electroconvulsive therapy
- Stroke
- Transient ischaemic attack (a 'mini stroke')
- Alzheimer's disease
- Brain surgery.

2.5 THEORY

Six theories of human amnesia are examined. Each is categorized according to the processing ability that is conceived to underlie the amnesic deficit. The theories fall into one of four categories: consolidation, retrieval, semantic encoding, and context encoding deficit theories. The recently proposed context encoding deficit theories are found to offer the most satisfactory account of the human amnesic syndrome. It is suggested that the other theoretical approaches are best viewed as special cases of these context encoding deficit theories

Multiple Trace Theory[22]

Hippocampus is involved in converting episodic to semantic (encoding). Once semantic, the memory is independent of the hippocampus. Retrieval of episodic memories requires the hippocampus. Predicts that episodic memory for all time periods should be disrupted, but only recent semantic memories should be impaired..

Motor Skills[22]

Old motor skills tend to be retained and new motor skills can be learned (procedural memory) but they lack knowledge that they have learned the new skill (no declarative memory for the procedure).Newly learned skills do not generalize.

Impaired Short term Memory[22]

Associated with Frontal Lobe Lesions Difficulty with contextual information - know facts, but not why they know them. Schacter et al. (1984) Presented facts Remembered facts, but not where they learned them. Often confabulated where they learned the facts.

Concussion Amnesia[22]

Most common cause of amnesia is Anterograde amnesia (difficult to form and retain new memories). Amnesia is sometimes not noticeable until a few days or even a week after an injury or accident. e.g, sports player who retains detailed memories of what was happening during a game before an injury, but who has no recollection of what happened subsequent to the incident. Generally, this is temporary.

Encoding (Consolidation) Deficit Theory[23]

Retrograde amnesia is that the disorder impaired consolidation of memories during a time prior to the diagnosis of Korsakoff's syndrome, so that what appeared to be RA was really the long-term effects of Antrograde Amnesia

2.6.SYMPTOMS[24]

The two main features of amnesia are:

- Difficulty learning new information following the onset of amnesia (anterograde amnesia)
- Difficulty remembering past events and previously familiar information (retrograde amnesia)
- False memories (confabulation), either completely invented or made up of genuine memories misplaced in time
- Confusion or disorientation

2.7. DIAGNOSIS[25]

In order to diagnose amnesia other possible causes of memory loss, such as Alzheimer's disease, dementia, depression, brain tumor, or epilepsy must first be ruled out by a physician. This is done through taking a person's medical history, interviewing family members, and through the use of diagnostic imaging tests such as magnetic resonance imaging (MRI) a computerized tomography(CT), or electroencephalogram (EEG)—which may identify damage to or abnormalities in the brain.

Other tests might include a lumbar puncture, a cerebral angiography, and various cognitive (psychometrics) tests. A Transient Ischemic Attack(TIA)—a mini or mild stroke—should also be investigated as a possible cause

In addition to investigating the patient's family history, triggering factors, (drug or alcohol use) and/or injury, a physician will perform a neurological exam which includes checking reflexes, sensory function, balance, and other physiological aspects of the brain and nervous system.

2.8.THE NEUROPATHOLOGY OF AMNESIA IN HUMAN[26]

Relations between brain damage and memory disturbance are outlined with emphasis on the so-called amnesic syndrome. Following a brief introduction into forms of memory and memory failures, the basic causes of brain damaage (with relevance to amnestic failures) are described. Thereafter, the two best-known forms of brain damage-amnesia relations are reviewed: the consequences of damage to medial temporal lobe structures and to diencephalic regions.

Other cases with more or less circumscribed damage to medial temporal lobe structures are reviewed so as to outline criteria for or against the hypothesis that there are regions within the medial temporal lobe whose damage might be critical for the amnesic syndrome. Two cases of diencephalic amnesia are summarized in particular as they have received extensive neuropsychological and neuropathological investigation. Other cases with, for example, Korsakoff's disease are reviewed, as well as cases with diencephalic, or combined mesencephalic-diencephalic damage without nutritional causes.

A third group of patients with massive, but still selective amnesic disturbances are then described: cases of basal forebrain damage, followed by descriptions of Alzheimer's disease which has similarities in the underlying neuropathology. This leads over to cases with more generalized intellectual deteriorations (dementia), which may have developed on the basis of primarily cortical damage or damage principally to basal ganglia structures.

After reviewing cases with mainly material-specific memory failures--usually as a consequence of restricted neocortical damage--a separate section follows on patients in whom retrograde amnesia is the prominent symptom. The contribution of animal models of human amnesia is critically reviewed and discrepancies are analyzed between human and animal memory disturbances. This section emphasizes the value of investigating inter-dependencies between brain structures by pointing out that relations between memory disturbances and brain damage may be more complicated than apparent from a simple structure-function assignment.

TABLE 1.-Selected Amnestic Disorders and Their Pathophysiology [27]

Disorder	Physiologic Substrate for Amnesia
Alzheimer's disease	• Neuronal loss in nucleus basalis of Meynert • Loss of acetylcholine and choline acetyltransferase activity in septohippocampal pathway • Neurofibrillary tangles in CA1 and CA3 pyramidal cell regions of hippocampus • Neuritic plaques in dentate gyrus • Neuronal loss in entorhinal cortex, layers 11 and IV
Korsakoff's syndrome	• Thiamine deficiency-induced lesions in dorsomedial nucleus of the thalamus, mamillary bodies, mamillothalamic tract
Transient global amnesia	• Transient ischemia to hippocampus and other medial temporal lobe regions
Head trauma	• Injury to hippocampus and other medial temporal lobe regions
Herpes simplex encephalitis	• Viral attack on limbic regions including medial temporal lobe and cingulate gyrus
Partial complex epilepsy	• Possible damage to CA3 region
Infarction, anoxia	• Damage to medial temporal lobes or thalamus

2.9.PATHOLOGY

Plaques And Tangles

There is two abnormal structures in their Alzheimer brains, both amyloid plaques and neuro fibrillary tangles. These are particularly common in regions of the brain that are very important in the creation of memory. Plaques consist mainly of dense, insoluble deposits of protein and cellular material that build up around and outside neurons. Tangles are twisted, insoluble fibres that develop inside nerve cells. While such structures can be found in limited numbers in the brains of many healthy elderly people, they are present to a much greater degree in those who have shown the symptoms of Alzheimer's disease.[28]

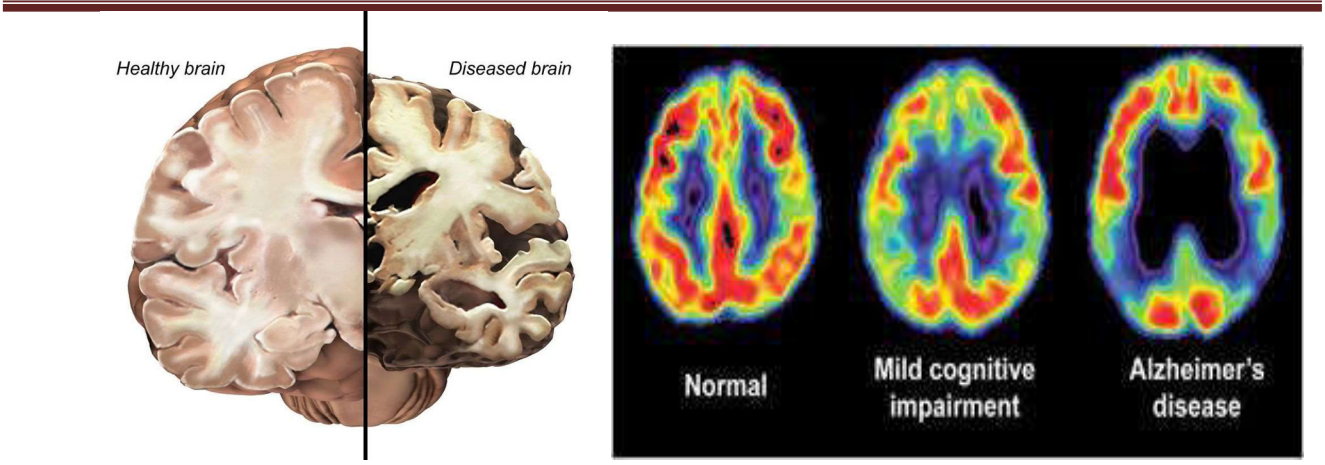


Figure 2 Cross sections and image of the brain show atrophy, or shrinking, of brain tissue caused by Alzheimer's disease.

Beta amyloid

In the normal brain, a larger protein the amyloid precursor protein is broken down into three smaller protein fragments known as alpha-amyloid, beta-amyloid, and gammaamyloid. In those individuals with Alzheimer's disease, when amyloid precursor protein breaks down it creates a disproportionate amount of beta-amyloid but less alpha-amyloid and gamma-amyloid protein than usual. This excess of beta-amyloid protein overwhelms the brain's capacity to remove it and so it accumulates as insoluble gum like plaques, which may also contain other molecules, neurons, and non-nerve cells.

Such plaques "gum up the works" by damaging the connection points that is the synapses, between neurons and, as a consequence, interfere with such cells' ability to communicate. In Alzheimer's disease, early plaques develop in the hippocampus, a brain structure involved in encoding memories, and also in other parts of the cerebral cortex that are necessary for thought and decision making. As the disease progresses, additional plaques form in the frontal lobes of the brain. The more severe the symptoms of Alzheimer's disease, the more plaques will typically be found in the patient's brain during autopsy. Such betaamyloid plaques also trigger an inflammatory response. Part of this process involves the creation of oxygen free radicals highly reactive molecules which can damage or kill other cells by creating holes in their membranes or binding to their DNA and interfering with survival. This plaque-related inflammatory process appears to destroy large numbers of brain cells in Alzheimer's patients and its effects are obvious in stained brain sections. This could be why taking anti-inflammatory drugs, for other health problems, may accidentally reduce the probability of developing Alzheimer's disease. [29]

Tangles

Tau is important protein,that normally binds with tubulin, which is used to form structures known as microtubules. These microtubules are of great biological significance because they act like the pillars and girders of a building, giving shape and structure to cells. In patients with Alzheimer's disease, tau becomes chemically abnormal and begins to pair with other threads of tau and become tangled. As this happens, neuron microtubules disintegrate. These "tangles" prevent the movement of nutrients and other molecules to the nerve endings of the neurons, and as a result, communication malfunctions can occur, often followed by cell death. Tangles initially interfere with the functions of the brain's temporal lobe, causing memory loss and difficulties in reading and writing. As plaques and tangles begin to appear in the frontal lobes, personality disorders and other symptoms appear. While tangles can be seen also in the brains of healthy older people, they are relatively rare. In Alzheimer's patients, the worse the symptoms, the more common tangles are usually found to be on brain autopsy.

Normally tau protein undergoes phosphorylation, the addition of a phosphate (PO4) group to a protein or to a small molecule. Phosphorylation provides a very fast way of regulating proteins. If a protein, is regulated by phosphorylation it is always present in "standby" mode. When an activating signal arrives, the protein is phosphorylated and then performs in the way intended .the tau in the brains of Alzheimer's patients is abnormal in that it is hyperphosphorlylated, that is phosphorlylated to excess. It has been corrupted by several extra molecules of phosphorus. As a result, the tau malfunctions and becomes unable to support tubulin's role in the production of microtubules, which, therefore, lack integrity and begin to twist. Communication and cell nourishment is compromised and eventually declines to zero. The neuron cannot be sustained and begins to wither. The cell membranes collapse and every part of the neuron disintegrates and with it synapses, each representing a memory fragment. [30]

Nucleus Basalis Of Meynert

Plaques and tangles do not seem to be the only cause of Alzheimer's related neuron death. Certain neurons found at the base of the brain known as the Nucleus Basalis of Meynert, appear to die in Alzheimer's disease without any evidence of the interference of plaques or tangles. Such neurons produce acetylcholine, a chemical messenger used in communication between neurons. Normally acetylcholine-manufacturing neurons have long branches that reach into the hippocampus and cortex and are thought to play an important role in learning and memory. Their death in Alzheimer's disease means that less acetylcholine is available to the brain, probably interfering with both its memory and learning capacity. [31]

Genetics

Alzheimer's disease seems linked, to some degree, to a gene in chromosome called the APO E gene, which codes for apolipoprotein E. This protein is involved in the cellular movement of cholesterol throughout the body. There are three slightly different types (known as alleles) of the APO E gene, namely APO E2, APO E3, and APO E4. Everybody has inherited two copies of this gene, one from each parent. The E3 variant is the most common and occurs in between 40 and 90 percent of the populations of particular regions; E2 and E4 are less common, being present in 2 percent and 6 to 37 percent of people respectively.It has been demonstrated that the probability of developing sporadic late-onset Alzheimer's disease is much higher in those possessing the EPO E4 allele. Indeed, anyone who has inherited copies of the APO E4 alelle from both parents has a 15 times greater risk of developing sporadic Alzheimer's disease than someone without this form of the APO E gene. Consequently, in Alzheimer's patients, carriers of the APO E4 are common, with this allele being present in approximately 40 percent. [32]

The Cholinergic System

Many of the cognitive deficits seen in Alzheimer's disease seem linked to problems in the cholinergic system, Choline acetyltransferase is reduced, especially in the temporal cortex and the hippocampus. In addition, Alzheimer's affected brains, again in their cortical and hippocampal areas, show a marked decrease in forebrain cholinergic neurons. Concentrations of cerebrospinal fluid acetylcholine are also low in Alzheimer's disease patients and are positively correlated with dementia scale test scores. All this evidence, combined with studies show that anticholinergic drugs cause a decline in memory, which support the view that many of the cognitive deficits that occur in Alzheimer's disease patients are probably caused by cholinergic abnormalities.[33]

It has been the prevailing view that the symptomatic efficacy of AChEIs is attained through their augmentation of acetylcholine-mediated neuron-to-neuron transmission. However, there is evidence that AChEIs may slow disease progression and hippocampal atrophy and may have disease modifying effects. In addition, symptomatic improvement in AD patients is not restricted to agents that enhance acetylcholine function in the brain, as is the case for memantine which acts on another neurotransmitter. Interestingly, memantine, whose benefits also appear to be best for moderate-to-severe AD, has been recently linked to modulation of inflammation also.[34]

The Catecholaminergic

The neurotransmitters of the nervous system dopamine and serotonin can be derived from the amino acid tyrosine. The enzyme tetrahydrobiopterin, required for the synthesis of these two neurotransmitters, is significantly depressed in the cerebrospinal fluid of patients suffering from Alzheimer's disease.

It is not surprising, therefore, that the brains of patients with this type of dementia contain less dopamine and serotonin than usual. Several studies have demonstrated that the subnormal production of these neurotransmitters appears to be linked to the death of dopamine receptors and noradrenergic and serotonergic neurons, in the cortex and elsewhere in the Alzheimer's brain.[35]

The loss of the D2 receptor-enriched modules in the brains of Alzheimer's patients contributed to disturbances in information processing that may be responsible for cognitive and noncognitive impairments. Among the others, dopaminergic system may play a relevant role in the mechanisms involved in learning and memory processes, showing strong synaptic interaction with acetylcholine in different brain areas.[36]

Most of the publications dealing with the role of serotonin receptors in AD focus on the possible interplay between the serotonergic system and the amyloid-mediated part of pathophysiology. This is mostly based on the experimental finding demonstrating that administration of selective serotonin reuptake inhibitors (SSRI) in mouse models of AD reduces the production of toxic amyloid proteins and amyloid plaques. The fact that SSRIs seem to inhibit the production of toxic amyloid species makes them a promising tool to slowdown the progression of AD. Whereas serotonin receptors can influence processing of Aβ via modulation of activity of corresponding secretases , the role of serotonin receptors in NFT formation have not yet been analysed. However, there is indirect evidence suggesting the involvement of the serotonergic system in tau hyper-phosphorylation.

An additional and even more intrigue connection between tau pathology and 5-HT receptors involves the receptor-mediated modulation of the Brain-derived neurotrophic factor (BDNF) concentration. It is known that BDNF attenuates tau and Aβ pathologies by activating the phosphatidylinositol 3 kinase/GSK-3β pathway. Activation of this pathway, which leads to the inhibition of GSK-3β activity, attenuates phosphorylation of tau and, consequently, reduces pathology [37]

2.10.EPIDEMOLOGY

The prevalence of dissociative amnesia is approximately 1.0–2.6% of the total world's population and the incidence of global transient amnesia is 2.9–10 per 100,000 cases every year.[38,39]

According to the previous study, approximately around 34 million people have suffered from Alzheimer's disease, and among them 5 ≥ million people from the United States have been diagnosed as

Alzheimer's disease patients.[40] In India, more than 4 million people have some form of dementia. being second largest country in world, India has less number of alzheimers patient. curcumin, commonly known as haldi in India, helps reduce the risk of Alzheimer's disease, a brain disorder that results in memory loss, personality changes and a decline in the thinking ability. These adverse impacts, scientists believe, are related to the death of brain cells and a breakdown of connections.[41] As per a study conducted by scientists from the US -based University of California, curcumin inhibits the accumulation of destructive beta amyloids inert substances responsible for Alzheimer's in the brain. The study, involving genetically altered mice, suggests curcumin is far more effective in inhibiting formation of the amyloids than drugs currently being tested to treat the disease. They assert the extensive use of curcumin is possibly why India has the lowest rate of the disease in the world about 4.4 times less among adults aged 70-79 than the rate in the us.[42]

2.11.MEDICINAL HERBS USED IN TREATMENT OF AMNESIA[43]

Three Herbs Showing Promising Memory Enhancing Capabilities Extensive basic research studies on amnesia have been conducted over the past few decades with the attempt to investigate the anti-amnesic therapeutic potentials of various herbs. Although a number of herbal medicines have been demonstrated with anti-amnesic effects, clinical trials on the use of herbal medicines for the treatment of amnesia have not yet been published. Despite the difficulty of new drug development, several research groups have discovered some herbs with promising efficacies in the clinical setting and great application potential in memory enhancement in healthy subjects, patients with dementia or AD which could be potential candidates for amnesia as well. Clinical data regarding trials of Centella asiatica, G. biloba and H. serrata in human beings are summarized below.

Centella asiatica (L.) Urb,

The cognitive-enhancing effect of C. asiatica was tested in 28 healthy elderly subjects in a randomized, placebo-controlled, double-blind study. Treatment with a high dose (750 mg) of C. asiatica repeatedly for two months increased the percentage accuracy of both numeric working memory and word recognition,

Ginkgo biloba L.

In a randomized, double-blind, placebo-controlled trial, healthy volunteered participants were randomly divided into two groups: Ginkgo (40 mg, thrice/day) and matching placebo for 6 weeks. There was no significant difference in standard neuropsychological tests of learning, memory, attention and concentration or naming and verbal fluency between the ginkgo and placebo groups

Huperzia serrata (Thunb.) Trev.

Huperzine A, derived from H. serrata, has been shown to have antioxidant and neuroprotective functions. The neuroprotective effects of huperzine A have been widely studied in AD patients especially in China. It has been suggested that this herb may be as effective as tacrine and donepezil in the symptomatic treatment of dementia. Clinical efficacy and safety of huperzine A has been demonstrated in AD patients in different randomized and placebo-controlled trials in China.

Angelica gigas Nakai, Angelica sinensis (Oliv.) Diels, Cnidium officinaleMakino. Ligusticum wallichiiFranch, Coptis chinensisFranch, corydalis yanhusuo, Cnidium monnieri (L.) Cuss, Desmodium gangeticum (L.) DC, Foeniculum vulgareMill, Apiaceae Gastrodia elata Bl, Orchidaceae Geissospermum vellosiiAllem, Ginkgo biloba L, Hypericum perforatumL, , Liuwei Dihuang Wang Lonicera japonicaThunb, Murraya koenigii (L.) Roxb, Nardostachys jatamansiDC, Nelumbo nuciferaGaertn, Paeonia lactiflora Pall, Panax ginseng C. A. Mey, Polygala tenuifoliaWilld,, Pueraria thunbergiana,Salvia miltiorrhiza Bge, Labiatae Salvia triloba L, Labiatae Schisandra chinensis(Turcz.) Baill, Scrophularia buergeriana Miquel, Scrophulariaceae Scutellaria baicalensisGeorgi, Teucrium polium L,Thespesia populneaMilo, Tremella fuciformisBerk are used in the treatment of amnesia induced by scopolamine through cholinergic system.

Acorus gramineusSoland, Uncariae ramulus et Uncus, Uncariae rhynchophylla(Miq.) Jacks, were experimented for amnesia induced by Ibotenic acid through cholinergic system.

Bacopa monniera (L.) Penn.syn, Salvia miltiorrhiza Bge, acted through GABA system when for diazepam induced amnesia

2.12.TREATMENTS

TABLE 2:Pharmacological [44]

Generic	Indication	Side effects
Piracetam	Neurocognitive impairment, memory decline,	Sleep disturbances, diahhorea
Oxiracetam	Aging mental impairment	Psychomotor excitability, sleep disorders
Aniracetam	Memory decline, neurodegenerative disorder	Agitation, Anxiety, Restlessness, Insomnia
Pramiracetam	Aging mental impairment, anxiety	Insomnia, Dysphoria Gastralgia, heartburn
Phenyl Piracetam	Mental function impairment CNS, Neurotic disorder	Sleep disturbances
Levitiracetam	Epilepsy	Somnolence, Fatigue, coordination difficulties, behavioural abnormalities

TABLE 3: Revised Clinical and Research Criteria[45]

Drug	Class/Function	Phase
Phosiphen [(+)-phenserine tartrate	Inhibitor of amyloid precursor protein (APP) synthesis	I
HPP854	Beta-secretase-1 (BACE) inhibitor	I
E2609	Beta-secretase-1 (BACE) inhibitor	I
Atomoxetine	Selective norepinephrine reuptake inhibitor (NRI)	II
Ladostigil	Combined reversible acetylcholinesterase-butyrylcholinesterase inhibitor, and irreversible monoamine oxidase B inhibitor,	II
LY2886721	Beta-secretase-1 (BACE) inhibitor	II
Levetiracetam	Exact mechanism unknown. Enhancement of glutamatergic excitatory synaptic transmission	II
NewGam 10% IVIG	Intravenous immunoglobulin (IVIG)	II
Insulin detemir	long-acting human insulin analogue	II
Curcumin	Exact mechanism unknown. Antioxidant and anti-inflammatory properties, decrease in Aβ	II
Simvastatin	HMG COA reductase inhibitor	IV
Rivastigmine(Exelon transdermal patch)	Reversible acetylcholinesterase-butyrylcholinesterase inhibitor	N/A

Nonpharmacology Treatment [46]

- Physical, emotional and social stimulation

  focus on different types of activities, art therapy, aromatherapy, music therapy

- Cognitive therapy approaches

  memory and orientation exercises

- Emotion-oriented interventions

  contact with animals, friends and family

- Caregiver training programs

  Caregiver training for family members
- Attention to safety
- Diet

2.13.METHODS TO INDUCE AMNESIA[47]

Electroshock Induced Amnesia

In the electroshock induced amnesic models retrograde amnesia is induced by the electric shock. The memory loss effect of this method is due to the electric shock rather than the convulsion.

Hypoxic Stress-induced Learning Deficits

Animals were housed in eight identical commercially designed chambers (30×20×20 in) that can accommodate six rats each and are operated under a 12-hour light–dark cycle (Oxycycler model A44XO; Reming Bioinstruments, Redfield, NY). Gas was circulated around each of the chambers, attached tubing, and other units at 60 L/min (i.e, one complete change per 30 s). The O2 concentration was continuously measured by an O2 analyzer and was changed throughout the 12 hours of light time (6:00 A.M. to 6:00 P.M.) by a computerized system controlling the gas valve outlets, such that the moment-to-moment desired oxygen concentration of the chamber was programmed and adjusted automatically. Deviations from the desired concentration were met by addition of N2 or room air (RA) through solenoid valves. For the remaining 12 hours of night time, oxygen concentrations were kept at 21%. This specific and validated

profile consists of 90 seconds of 10% O2 alternating with 90 seconds of RA for 12 hours during the light phase, and typically results in nadir PaO2 of 37 to 42 mm Hg and oxyhemoglobin saturations ranging from 68 to 76%. Ambient CO2 in the chamber was periodically monitored and maintained at 0.03% by circulating the gas through soda lime. The gas was also circulated through a molecular sieve (Type 3A; Fisons, East Grinstead, UK) to remove ammonia. Humidity was measured, and was maintained at 40 to 50% by circulating the gas through a freezer and silica gel. Ambient temperature was kept at 22 to 248C. After 12 weeks of either NA or PA, rats were started on the IH protocol for 14 days, after which water maze experiments were initiated and IH exposures continued until completion of all maze procedures.

Pharmacological & Discrimination Assays

Scopolamine-Induced Amnesia:

The administration of the antimuscarinic agent scopolamine to young human volunteers produces transient memory deficits. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in any behaviour task. The ability of a range of different cholinergic agonist drugs to reverse the amnesic effects of scopolamine is now well documented in animals and human volunteers.

Sodium Nitrite Induced Amnesia:

Sodium nitrite induced amnesia is a type of Interoceptive aversive stimuli model. The manipulation of brain metabolism was used to show the beneficial effects of substances which influence learning and memory. during investigations of sodium-nitrite (NaNO2) on brain metabolism, demonstrated a close relationship between oxidative metabolism and cholinergic function.

Other Chemical Induced Learning And Memory Impairment

Other chemical agents e.g Dizocilpine, anisodine, Clonidine, Triazolam, Clozapine Lignocaine Aluminium Ethanol, Carbon dioxide has been reported for the induction of various serious learning and memory impairment. Dizoclipine, a NMDA receptor antagonist has been reported for the memory impairment by blockadge of glutamate receptor. Anisodine (10m]7g/kg) also reported for significantly induction of memory dysfunction in mice, the latencies of the step down test ant step through test were significantly shortened, and errors of response in the step down test and step through test increased. The activation of α2-adrenoceptors has been reported to impair memory functions in both rats and humans. The α2-adrenoceptor subtype responsible for this detrimental effect is still unknown. The effect of the α2-agonists clonidine and guanabenz on memory processes, in dependence to the time of administration, was evaluated in the mouse

passive avoidance test. Clonidine (0.02–0.2 mg kg−1 i.p.) and guanabenz (0.1–0.3 mg kg−1 i.p.) induced amnesia in a dose-dependent manner . Aluminium, selenium, lead are neurotoxic metals which are also reported for the memory impairment. An infusion of 5 μm aluminium (as aluminum tartrate) showed memory deficts.

2.14.DRUG PROFILE

SCOPOLAMINE[48]

Scopolamine, an anti-muscarinic agent, competitively antagonizes the effect of acetylcholine on the muscarinic receptors by occupying postsynaptic receptor sites with high affinity and increases AChE activity in the cortex and hippocampus. Scopolamine diminish cerebral blood flow due to cholinergic hypofunction. Scopolamine additionally triggers ROS, inducing free radical injury and an increase in a scopolamine-treated group brain MDA levels and deterioration in antioxidant status. Scopolamine induces neuro-inflammation by promoting high level of oxidative stress and pro inflammatory cytokines in the hippocampus. Scopolamine is proven to increase levels of APP and Tau. Administration of scopolamine led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration.

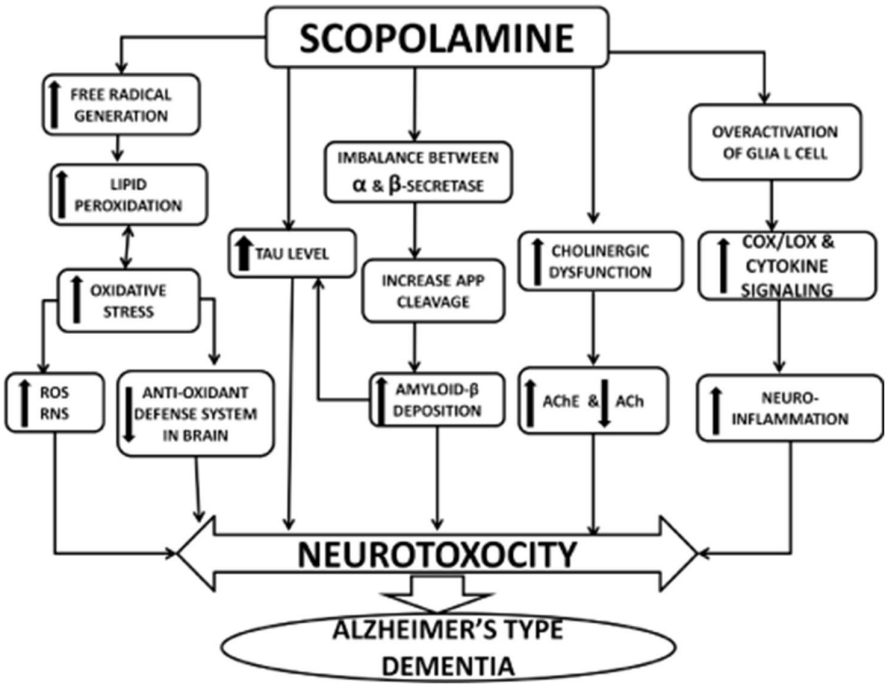


Figure 3 Mechanism of Scopolamine

2.15.PIRACETAM[49]

Piracetam a cyclic GABA derivative is the first representative of a new class if psychotropic drugs named as nootropic agents. These drugs were shown to improve learning acquisition and retention of learning (memory) both in normal animals and in conditions of impaired cognitive functions. These effects were also noted clinically when piracetam and related compounds, were found to improve memory deficits in children and in geriatric individuals, as well as in hypoxic amnesia. And in cognitive deficits following acute traumatic brain damage and chronic alcoholism. Interestingly, piracetam and other nootropic agents have, even in large doses, virtually no effects on autonomic functions, psychomotor behaviour, limbic lobe activity and arousal. The mechanism of action of nootropic agents remains equivocal, despite extensive neurochemical studies and demonstrated effects on some neurotransmitter system, including monoamines and prostaglandins. There is extensive evidence linking the central cholinergic system to memory and the data has been reviewed, cognitive dysfunction has been shown to be associated with impairment of cholinergic function and facilitation of central cholinergic activity improves learning and memory.

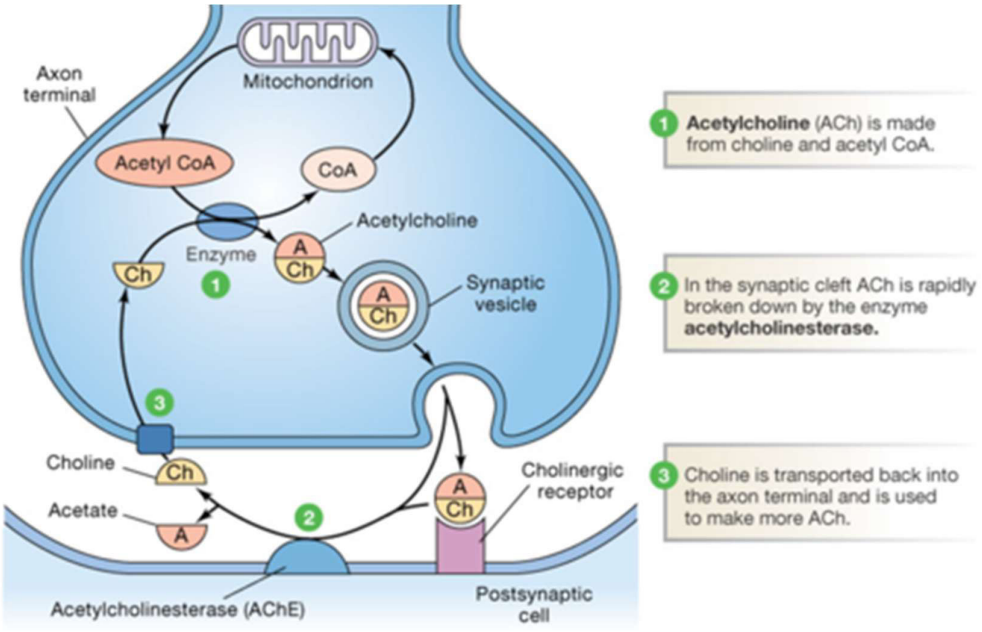


Figure 4 Mechanism of Piracetam

3.PLANT PROFILE[50]

Taxonomy:

Botanical name: *Citrullus lanatus* (Thunb)

Class: Equisetopsida

Kingdom: Plantae

Genus: Citrullus

Family: Cucurbitaceae

Order: Cucurbitales

Vernacular names:

Common name: Watermelon, Wild Watermelon

Local name: Tarbooz

English: Watermelon

Marathi: Tarbooz, Kalingad

Bengali: Tormuz

Malayalam: Thannimathan

Kanada: Kallagadi

Assamese: Tarmuj

Telugu: Pendalam

Tamil: Kizhangu

Figure 5: Whole Plant

Figure 6: Fruit

3.1.Botanical Description:

The watermelon is a large annual plant with long, weak, trailing or climbing stems which are five-angled (five-sided) and up to 3 m (10 ft) long. Young growth is densely woolly with yellowish-brown hairs which disappear as the plant ages. The leaves are large, coarse, hairy pinnately-lobed and alternate; they get stiff and rough when old. The plant has branching tendrils. The white to yellow flowers grow singly in the leaf axils and the corolla is white or yellow inside and greenish-yellow on the outside. The flowers are unisexual, with male and female flowers occurring on the same plant (monoecious). The male flowers predominate at the beginning of the season; the female flowers, which develop later, have inferior ovaries. The styles are united into a single column. The large fruit is a kind of modified berry called a pepo with a thick rind (exocarp) and fleshy center (mesocarp and endocarp). Wild plants have fruits up to 20 cm (8 in) in diameter, while cultivated varieties may exceed 60 cm (24 in). The rind of the fruit is mid- to dark green and usually mottled or striped, and the flesh, containing numerous pips spread throughout the inside, can be red or pink (most commonly), orange, yellow, green or white.[51]

Citrullus lanatus (Thunb.) Matsum. and Nakai, commonly known as watermelon, belonging to the family *Cucurbitaceae* is native to India. It is found in forest lands, riversides, and wasteland, and also gets cultivated on a large scale. It is an excellent source of the arginine, Vitamin A, B and C, carotenoids, lycopene, carbohydrates, sodium, magnesium, potassium, and water[52]

3.2.Morphological characters[53]

Stems: *Citrullus lanatus*(Thunb.) is a prostrate or climbing annual with several herbaceous, rather firm and stout stems up to 3 m long; the young parts are densely woolly with yellowish to brownish hairs while the older parts become hairless.

Leaves: The leaves are simple, alternate on long petioles, cordate with seven shallow lobes and variously serrated margins, very hairy on the abaxial surface, acute, deep green, and about 7 – 15 cm in diameter. Tendrils are normal and spiral.

Flower: Male and female flowers grow on the same plant. Male flowers are found in clusters and appear before the female flowers. Both have yellow petals, five in number, and sepals, also five in number and greenish in color. Occasional hermaphrodite flowers are produced. Fruits: The fruits are globular with

shallow grooves, about 14 –20 cm long. The skin is greenish yellow. The flesh is almost white/light yellow, sweet.

Seeds: The seeds are small, light brown white and smooth, between 0.4 and 1.1cm long and 0.2 – 0.3cm wide.

3.4.Traditional claims:

Citrullus lanatus seeds are used as anthelmintic, anticancer, antibacterial, demulcent, relieves, constipation, diarrhea, cardiac, diuretic, kidney troubles, cooling effect, demulcent, pectoral, tonic, burns, swellings, rheumatism, gout [54,56,58]. The *Citrullus lanatus* and its products are used as anti-inflammatory, laxative, antihypertensive, and antidepressant. The Mateerauseto eradicate the urinary problems, weakness[55.] The pulp of with strawberry, peach, pine apple, and cucumber pulp. *Citrullus lanatus* fruit used in masks for dry skin[57].The rind of fruits is used in treatment of alcoholic poisoning and diabetes. The root is used as purgative and in large doses as an emetic. Seeds are also used as vermifuge and have hypertensive action. Fatty oils in the seeds as well as in aqueous and alcoholic extracts paralyze tapeworms and roundworms [58]. It is also used for cleansing and purifies kidney and bladder, for treating erectile dysfunction and hepatomegaly and jaundice[59,60]

4.PLANT LITERATURE REVIEW:

Antibacterial activity and antifungal activity:

Braide W et al reported that crude extract of watermelon seeds using hot water, cold water, methanol and ethanol showed the antimicrobial activity.[61]

In another study, Thirunavukarasu et al reported that the ethanolic extract of crude extract of *citrullus colocynthis, citrullus lanatus* and *citrullus vulgaris* was found more effective than respective aqueous and chloroform extract against bacteria and some fungal strains[59]

Antimicrobial activity:

Ahmed Hassan et al reported the antimicrobial effects of chloroform of *Citrullus lanatus* extracts against bacteria suggest that, different parts of plant possess remarkable therapeutic action that can support the traditional usage of this plant in the treatment of bacterial and fungal diseases such as gastrointestinal infection, diarrhoea, respiratory and skin diseases.[60]

Sathya et al, reported antimicrobial activity of methanol extract of *Citrullus lanatus* seed was carried out against 10 bacterial species and 5 fungal species.[62]

Antiulcerative activity:

Gill et al, reported that methanolic extract of *Citrullus lanatus* seeds showed maximum antioxidant potential and was evaluated for its anti-ulcerogenic activity by Pyloric Ligated and Water Immersion Stress induced ulcer models in rat[63]

Lucky et al, reported seeds of *Citrullus lanatus* possesses significant antiulcer activity in animal model. [64]

Bharadwaj et al reported the antiulcer activity of crude methanolic extract of *Citrullus lanatus* seeds in two different ulcer models in albino Wistar rats was also studied.[65]

Antioxidant activity:

Rahman et al reported the In-vitro anti-oxidant activity of the n-Hexane, Chloroform and Ethanol extract of *Citrullus lanatus* seeds were studied. The order of possessing antioxidant activities were n-hexane>ethanol>chloroform extracts of *Citrullus lanatus* seeds[66]

Anti-inflammatory activity:

Madhavi et al reported that *Citrullus lanatus* seed oil is extracted with nhexane and tested for in-vivo and in-vitro antiinflammatory activity. The oil was screened for in-vivo anti-inflammatory activity by Carrageenan-induced paw edema in rat model and In-vitro antiinflammatory activity by human red blood cell membrane stabilization method.[67]

Deng et al reported in another study, mouse model with ear edema induced by xylene and the rat model with paw edema or granuloma by carrageenan or cotton pellet were used for anti-inflammatory effect of the *Citrullus lanatus* aqueous extract.[68]

Gastroprotective Activity:

Sharma et al reported the gastroprotective potential of *Citrullus lanatus* fruit pulp aqueous extract (CLE) on pyloric ligation and indomethacin induced ulcer model in wistar albino rats was evaluated.[69]

Activity against prosthetic hyperplasia:

Olamide et al reported the administration of methanolic extract of *citrullus lanatus* creduced the prostate size and a potential candidate in management of androgen dependent conditions like benign prostate hyperplasia[70]

Laxative Activity:

Sharma et al reported the effects of the aqueous fruit pulp extract of *Citrullus lanatus* and reference standard on the gastro intestinal motility rate were also evaluated. the aqueous fruit pulp extract of *Citrullus lanatus* has a significant laxative activity[71]

Antigiardial Activity:

Hassan et al reported Antigiardial activities of *Citrullus lanatus* var. citroides fruits, petroleum ether, ethyl acetate, butanol crude extracts as well as Cucurbitacin E and Cucurbitacin L 2-O-β-glucoside pure isolated compounds from *Citrullus lanatus* var. citroides was investigated and revealed to have strong potent antigiardial activity, may be recommended as new source for the treatment of giardiasis[72]

Anti-hepatotoxic activity:

Altas et al reported the ability of watermelon juice to protectagainst CCl4-induced hepatotoxicity and oxidative stress[73]

Anti-atherosclerotic activity:

Poduri et al reported administration of C. lanatus 'sentinel' extract attenuated atherosclerosis in both arch and thoracic regions of aortas in mice. It is possible that citrulline in C. lanatus 'sentinel' extract may contribute to reducing atherosclerosis[74]

Anti-secretary activity:

Oluwole et al reported the effects of the juice of *Citrullus lanatus* was evaluated on gastric acid secretion and pH in Indomethacin-induced ulceration in male albino rats.[75]

Analgesic activity:

Kumari et al reported the analgesic activity of aqueous extract of *Citrullus lanatus* peels.[76]

Anti-diabetic activity

Jiyun et al reported the anti-diabetic potential of watermelon (*Citrullus vulgaris* Schrad) was evaluated in vivo using ICR mice, which suggest that watermelon has a beneficial effect on diabetes[77]

Mercury chloride intoxication:

Owoeye et al reported, neuroprotective potential of *Citrullus lanatus* seed extract and vitamin E on mercury chloride intoxication on the frontal cerebral cortex of male rats were studies.[78]

Urolithiatic And Diuretic activity:

Siddiqui et al reported the study validated the traditional uses of *Citrullus lanatus* and demonstrated that pulp extract possessed significant anti-urolithiatic and diuretic activities in *vivo* and in *vitro* in male Wistar rats[79]

Reproductive Functions And Antioxidant Activities:

Daramola et al reported the effects of aqueous extract of *Citrullus lanatus* fruit on reproductive functions and antioxidant activities in arsenic-treated male Wistar rats and provides protection for sperm cells against arsenic-induced oxidative stress[80]

Hepato- and neuro-protective Effect:

Oyenihi et al, Evaluated the potential protective effects of watermelon juice on ethanol-induced oxidative stress in the liver and brain of male Wistar rats[81]

5. SCOPE OF THE WORK

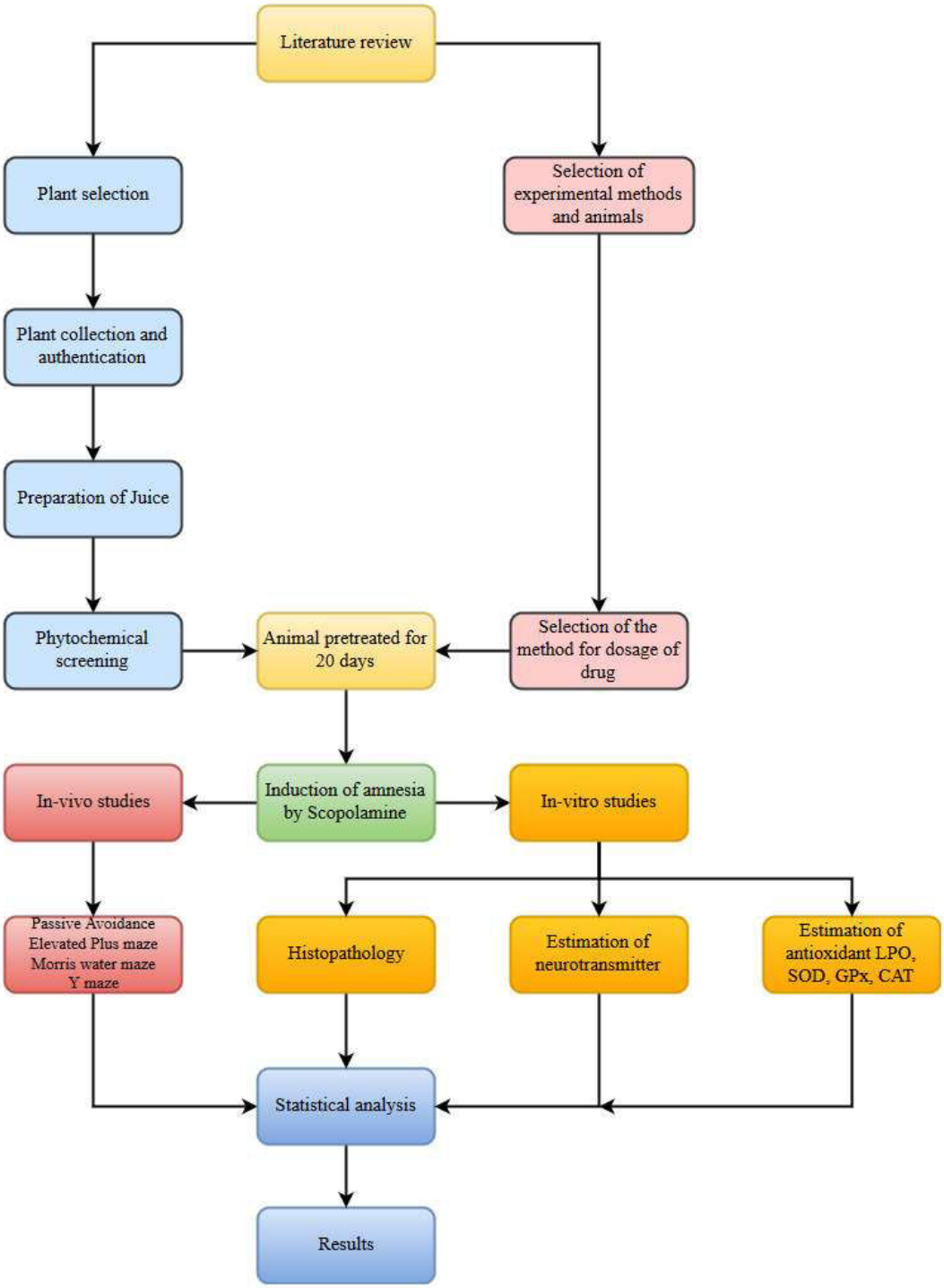
Since ancient times, herbal medicines have been documented and used for nootropics (cognition-enhancing agents which aim at improving concentration, memory retention and problem-solving ability). Over the years, scientists have exerted great efforts to identify new anti-amnesic compounds from herbal medicines to meet the unmet medical needs.

Potential new drugs from herbal medicines may be relevant in the treatment of cognitive disorders including amnesia. Herbal medicines can not only act synergistically with other components from the same herb, but also enhance the activity of active components from other herbs in accordance with traditional practices including traditional and Ayurvedic medicines.

No conventional or alternative therapy is currently available to cure amnesia. Current therapeutic strategies for amnesia are mainly focused on enhancing or restoring cerebral circulation, restoring the level of neurotransmitters including acetylcholine (ACh), scavenging free radicals and restoring cell membrane fluidity. In the management of amnesia as well as AD, sustained treatment with cholinesterase inhibitors including piracetam,, donepezil, rivastigmine, galantamine and tacrine have been used. However, these drugs have questionable efficacy and may induce severe side effects including nausea, vomiting, diarrhea and muscle cramps.

The present study is to prove the memory enhancement and cognitive effect of *citrullus lanatus* (Thunb.) on scopolamine induced amnestic swiss albino mice using various memory retention experiments such as Y maze , Morris water maze, Passive avoidance, Elevated Plus Maze and estimation of Acetylcholinesterase and antioxidants.

6. PLAN OF WORK



7. MATERIALS AND METHODS

7.1. Collection and Authentication:

Watermelon fruits (green skin, red flesh) were procured from a fruit vendor in a local market in Chennai,Tamilnadu,India The plant material was identified and authenticated by Prof P.Jayaraman, Ph.D, Director: Professor, Presidency College, and Chennai 600005. Plant Anatomy Research Centre. Tambaram, Tamil Nadu, Chennai-600045. Reg.No PARC/2017/3433. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97

7.2. Preparation of Aqueous Extract of *Citrullus lanatus* (Thunb.) (AECL)

Watermelon skin was peeled and the seeds removed. The mesocarp of the ripe fruit was chopped into thin slices and crushed to juice with a blender.

7.3. Method of Preparation of AECL:

The watermelon juice obtained was filtered through a fine mesh muslin cloth to get the fresh watermelon fruit juice. This is 100% concentration. A 25% and 50% concentration was prepared by diluting a pure watermelon juice with filtered tap water in the ratio of (1 : 3 and 1 : 1) (v/v)[81,82] .Fresh Juice was prepared on daily basis for the study.

7.4. Experimental Animals:

A total of 30 Swiss Albino Mice (25–30 g) were procured from the Kings Institute of Sciences, Chennai, Tamilnadu, India for the study. Animals were maintained at 25 ± 2°C and kept in well ventilated animal house under natural photoperiodic condition in polypropylene cages with paddy husk as bedding with free access to food and water *ad libitum.* The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by Institutional Animal Ethical Committee (IAEC) of C.L. Baid Metha College of Pharmacy, Rajiv Gandhi salai, Old Mahabalipuram Road, Jyothi nagar, Thoraippakam, Chennai -600 097, Tamil Nadu, India

Reg No:321/PO/Re/S/01/CPCSEA

7.5. PHYTOCHEMICAL ANALYSIS: [83]

The *Citrullus lanatus*(Thunb.). was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

I. Test for alkaloid:

The extract was treated with dilute hydrochloric acid and filtered. The filtrate was used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d**) Wagner's test (Iodine-Potassium Iodide Solution)**

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

II. Test for Carbohydrates

a) Molisch's test:

The extract was treated with 3ml of alpha-napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test ($CuSO_4.7H_2O$+KOH+Potassium Tartartes):

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + $CuSO_4.7H_2O$)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non- reducing sugars.

III. Test for steroids

a) Liberamannburchard test:

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids

IV. Test for proteins

a) Biuret's test:

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test:

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

V. Test for Tannin's

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

VI. Test for Phenols

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

VII. Test for Flavonoid

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavonoids.

VIII. Test for Gums and Mucilage

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

IX. Test for Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the yjunction of two liquids indicates the presence of glycosides.

X. Test for Saponins

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

XI. Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

XII. Test for sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

7.6. EXPERIMENTAL DESIGN:

On the first day of experiment the animals were divided randomly into six groups of five animals each. Amnesia is induced by single dose of scopolamine i.p for the II, III, IV, V and VI groups were performed on the 21st day of the pre-treated animals. Control animals were given water ad libitum.

Grouping

Mice were divided into 6 groups of 5 animals each.

Group I – Normal Water *ad libitum*

Group II – Normal water *ad libitum* + On the 21st day Scopolamine was injected (1mg/kg,i.p.)

Group III-Normal water *ad libitum* + Piracetam (200mg/kg, i.p.) injected for 20 days + On the 21st day Scopolamine was injected (1mg/kg,i.p.)

Group IV, V and VI- 25%, 50% and 100% AECL respectively *ad libitum* for 20days + On the 21st day Scopolamine was injected (1mg/kg.,i.p.)

7.7. METHOD EMPLOYED FOR EVALUATION OF MEMORY ENHANCING ACTIVITY IN MICE:

7.7.1.Passive-avoidance test[PA][84]

The apparatus consisted of a box (27x27x27 cm) having three walls of wood and one wall of Plexiglass,featuring a grid floor (3mm stainless steel rods set 8mm apart) with a wooden platform (10x7x1.7cm) in the centre of the grid floor. Electric shock (20V,A/C) was delivered to the grid floor. During training session ,each mouse was gently placed on the wooden platform set in the centre of the grid floor, when the mouse stepped down and placed all its paw on the grid floor, shocks were delivered for

15seconds and the Step Down Latency(SDL) was recorded. SDL was defined as time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range of 2 to 15 seconds during the first were used for the second session and the retention test. The second session was carried out 90minutes after the first test. During second session, if the animals stepped down before 60seconds, electric shocks were delivered once again for 15seconds. During the second test, animals were removed from shock free zone ,if they did not step down for a period of 60seconds and subjected to retention test. On the 20th day, after the treatment of last dose training was given and memory retention was examined after 24 hours (i.e, on 21st day) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300 seconds.

7.7.2 Elevated Plus Maze[EPM] [85,86,87]

The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (i.e, 20th day of drug administration) for each animal. If the animal did not enter into one of the covered arm within 90 sec, it was gently pushed into one of the two covered arms and TL was assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task memory was examined 24h (21st day).

7.7.3. Morris Water Maze [MWM] [88]

Spatial learning and memory were evaluated by the Morris water maze. The procedure was to perform place navigation test from day 1 to 4, in which the escape latency (EL) (the time required to escape onto the hidden platform) was used to evaluate learning and memory function. Mice that found the platform were allowed to remain on the platform for 20 s and were then returned to the home cage. If mice did not reach the platform within 120 s, it was gently guided to the platform by the experimenter, where it remained for 20 s. The last trial was regarded as the probe test on day 5 after removal of the platform which was performed to test the ability of mice to find the removed platform by memory

7.7.4 Y maze Test [YM] [89]

The behavioural test was conducted in a large quite room. A stop watch was used to score the behaviours and all events were observed manually. A Y-maze is made up of three equally spaced arms,

labelled as A, B, and C which are 120° from each other, 41 cm long and 15 cm high. It was used to assess the spontaneous alternation in the mice. The floor of the apparatus is 5 cm wide and is levelled with saw shaves. Each mice was stationed in one of the arms and allowed to freely explore the apparatus. The sequence or consecutive entrance of the animals into the arms is termed an alternation. The total number of arms entered minus two is termed spontaneous alternations, and the percentage alternation was calculated as {(actual alternations/maximum alternations) × 100}. 5 min was assigned as the test time limit for each of the animals in the Y-maze apparatus. Recorded data is the total arm entries indicate the total number of a single arm entered (e.g. ABCBCABACBC, contain 11 entries), from which the correct and wrong alternations are recorded.

7.8. BIOCHEMICAL ESTIMATION

7.8.1. Collection of Brain Sample

After behavioural testing (retrieval) by screening models of memory, the animals were sacrificed by cervical dislocation. The whole brain was carefully removed from the skull and weighed. 10% w/v brain homogenate was then prepared by homogenizing it in ice-chilled phosphate buffer (pH 8, 0.1M). The homogenate was subsequently centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min, and the supernatant was separated and used for the neurotransmitter and antioxidants estimation.

7.9.ESTIMATION OF ANTIOXIDANT

7.9.1. Estimation of Lipid Peroxidation(LPO): [90]

Lipid peroxidation was estimated by the method of Ohkawa et al. (1979). The pink colored chromogen formed by the reaction of 2-thiobarbituric acid (TBA) with breakdown products of lipid peroxidation was read at 532 nm.

Reagents

1. 8.1% sodium dodecy sulphate (SDS)
2. 20 % acetic acid
3. 0.5% TBA
4. N-Butanol and pyridine (15:1 v/v)
5. Stock melondialdehyde solution-1,1,3,3 - tetramethoxy propone (184 ug/ml)

Procedure

To 0.2 ml of sample, 0.2 ml of 8.1 % SDS, 1.5 ml of 20 % acetic acid, solution (adjusted to pH 2 to 3.5 with NaOH) and 1.5 ml of 0.8 % aqueous solution of TBA were added. The mixture was made up to 4.0 ml with distilled water and then heated in a water bath at 95°C for 60 minutes. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine were added and shaken vigorously. After centrifugation at 4000 g for 10 minutes, the organic layer was removed and its absorbance at 532nm was measured.

LPO expressed as nmol of MDA/min/mg protein.

7.9.2. Estimation of Suproxide Dismutase SOD:[91]

The assay of SOD is based on the inhibition of the formation of NADH- phenazinemethosulphate - nitroblue tetrazolium formazon. The color formed at the end of the reaction can be extracted into n-butanol layer and measured at 560 nm.

Reagents

1. Sodium pyrophosphate buffer
2. Absolute ethanol
3. n-butanol
4. Phenazine methosulphate (PMS)
5. Nitroblue tetrazolium (NBT)
6. NADH

Procedure

The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate and 0.3 mL of nitroblue tetrazolium, 0.2mL enzyme preparation and water in a total volume of 2.8 mL. The reaction was initiated by the addition of 0.2 mL NADH.The mixture was incubated at 30 C for 90 sec and arrested by the addition of 1ml of glacial acetic acid. The reaction mixture was shaken with 4 mL of n-butanol. The mixture was allowed to stand for 10 min and centrifuged. The intensity of the chromogen in the n-butanol layer was measured at 560 nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% incubation of NBT reduction in one minute. SOD activity was expressed as Unit/min/mg of protein .

7.9.3. Estimation of Glutathione Peroxidase (GPx) [92]

The activity of GPx was measured by the method of Rotruck et al. (1973). A known amount of enzyme preparation was allowed to react with H2O2 in the presence of GSH for a specified time period. Then the remaining GSH content was measured.

Reagents

1. Tris buffer: 0.4 M, pH 7.0
2. Sodium azide solution: 10 mM
3. TCA : 10%
4. EDTA : 0.4 mM
5. H2O2 solution : 0.2 mM
6. Glutathione solution: 2 mM

Procedure

To 0.2mL of tris buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide, 0.1 mL of sample were added. To the mixture, 0.2 mL of GSH followed by 0.1 mL of H2O2 was added. The contents were mixed well and incubated at 37 C for 10 min, along with a control containing all reagents except homogenate. After 10 min, the reaction was arrested by the addition of 0.5 mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% sodium nitrate)

GPx expressed as Units/min/mg of protein.

7.9.4. Estimation of Catalase(CAT) [93]

The activity of catalase was assayed by the method of Sinha (1972).

Dichromate in acetic acid was reduced to chromic acetate, when heated in presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split H2O2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H2O2 was determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Reagents

1. Phosphate buffer, 0.01 M, pH 7 2.
2. Hydrogen peroxide, 0.2 M
3. Potassium dichromate, 5%
4. Dichromate acetic acid reagent: Potassium dichromate and glacial acetic acid was mixed in the ratio 1:3. From this 1 ml was diluted again with 4 ml of acetic acid
5. Standard hydrogen peroxide, 0.2 mM

Procedure

To 0.9 ml of phosphate, 0.1 ml of plasma and 0.4 ml of H2O2 added. The reaction was after 15, 30, 45 and 60 seconds by adding 2 ml of dichromate acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and the colour developed was read at 530 nm. Standards in the concentration range of 20-100 mmoles was processed for the test. The activity of catalase was expressed as mmoles of H_2O_2 Utilised / second.

7.10. Estimation of Neurotransmitter

7.10.1. Estimation of Acetylcholinesterase(AChE):[94]

Acetylcholinesterase enzyme activity was estimated by Elman method.

Reagents

1. 0.1M phosphate buffer
2. DTNB Reagent
3. Acetylthiocholine (ATC)

Procedure

The mice were decapitated,brains are removed quickly and placed in ice cold saline. Frontal cortex, hippocampus and septum are quickly dissected out on a petri dish chilled on crushed ice.the tissues were weighed and homogenized in 0.1M phosphate buffer(pH 8).0.4ml aliquot of the homogenate is added to a cuvette containing 2.6ml of phosphate buffer(0.1M,pH 8) and 100µl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412nm in a spectrophotometer. When absorbance reaches a stable value, it is recorded as basal reading.20µl of substrate i.e, acetylthiocholine is added and change in absorbance is recorded. Change in absorbance/min is thus determined.

Calculations

The enzyme activity is also calculated by using the following formula

$$\text{Acetylcholinesterase activity (M/ml)} = \frac{\text{A/min X Vt}}{\varepsilon \text{ X b X VS}}$$

where, A/min= Change in absorbance per min

ε= 1.361x 104 M/cm

b= path length (1cm)

Vt= total volume (3.1ml)

VS= sample volume (0.4ml)

The final reading of enzyme activity is expressed as μ moles/min/mg tissue

$$\mu \text{ moles/min/mg protein} = \frac{\mu \text{ moles/ml sample}}{\text{mg protein/ml sample dilution}}$$

7.11. METHODS FOR HISTOPATHOLOGICAL STUDY[95]

The mice from each group were anaesthetized using inhalation of chloroform. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice cold normal saline and fixed in 10% formalin saline.

Paraffin embedded sections were taken 100μm thickness and processed in alcohol-xylene series and stained with Haematoxyli-Eosin dye. The sections were examined microscopically for histopathological changes in the cortex zone

7.12. STATISTICAL ANALYSIS

Data were analyzed using one-way ANOVA and expressed as mean ± standard deviation. Statistical analyses were performed using Graph Pad Prism version 7.04, for windows (Graph Pad software, San Diego, CA). Differences between mean values of different groups were considered statistically significant at *-P <0.05, **-P<0.01,***- P<.0001, ns-Non significant

8. TABLES AND GRAPH

Table 4 : Phytochemical screening of *Citrullus lanatus*(Thunb.)

S.NO	CONSTITUENTS	REMARKS
1	Alkaloids	Present
2	Carbohydrates	Absent
3	Protein	Absent
4	Steroids	Present
5	Phenols	Present
6	Tannins	Present
7	Flavonoids	Present
8	Gums and Mucilage	Absent
9	Glycosides	Present
10	Saponins	Present
11	Terpenes	Absent
12	Sterols	Absent

EFFECT OF AECL ON PASSIVE AVOIDANCE

Table 5

S.no	Groups	Transfer Latency (Sec)
1	Group I	43.83±1.24
2	Group II	$26.50\pm0.53a^{***}$
3	Group III	$39.50\pm0.74a^{ns}b^{***}$
4	Group IV	$32.03\pm1.31a^{***}b^{ns}c^{**}$
5	Group V	$35.33\pm1.53a^{**}b^{***}c^{ns}$
6	Group VI	$38.59\pm1.85a^{ns}b^{***}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

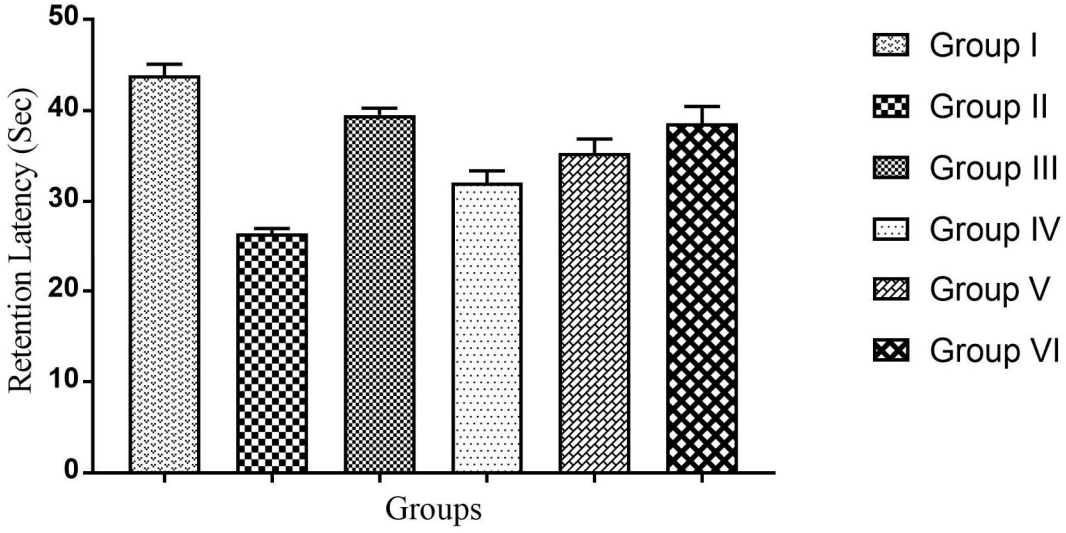


Figure 7

EFFECT OF AECL ON ELEVATED PLUS MAZE

Table 6

S.no	Groups	Transfer Latency (Sec)
1	Group I	31.21±1.04
2	Group II	$65.53\pm1.12a^{***}$
3	Group III	$18.01\pm1.56a^{***}b^{***}$
4	Group IV	$38.53\pm0.60a^{**}b^{***}c^{***}$
5	Group V	$25.94\pm1.74a^{ns}b^{***}c^{**}$
6	Group VI	$40.43\pm1.29a^{***}b^{***}c^{***}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

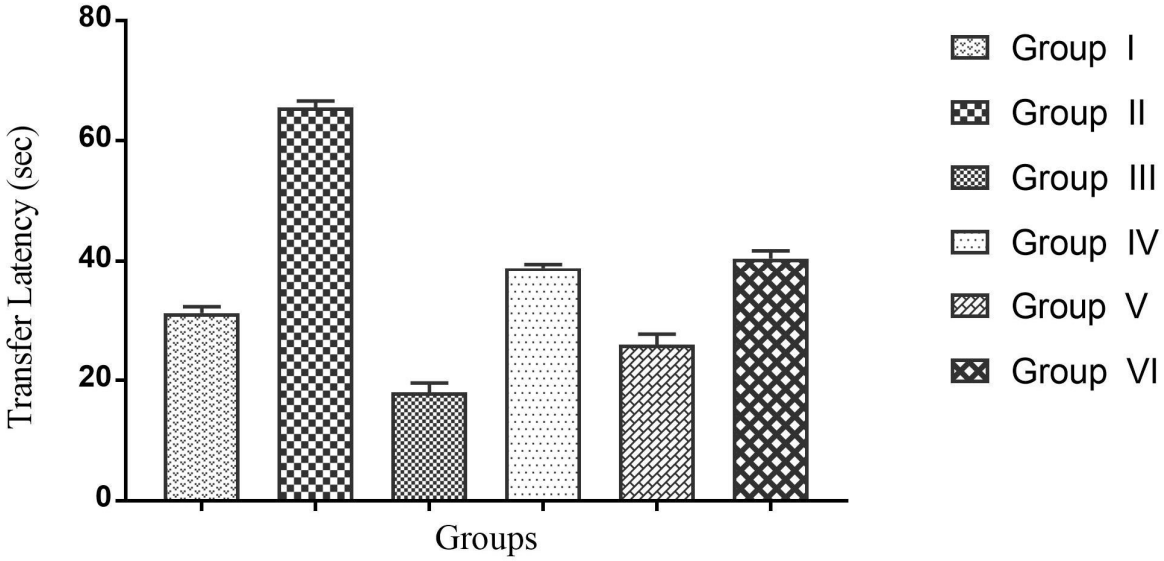


Figure 8

EFFECT OF AECL ON MORRIS WATER MAZE

Table 7

S.no	Groups	Escape Latency (Sec)
1	Group I	10.22±0.96
2	Group II	$6.08\pm1.20a^{**}$
3	Group III	$16.12\pm0.44a^{***}b^{***}$
4	Group IV	$12.65\pm0.57a^{ns}b^{***}c^{*}$
5	Group V	$14.09\pm0.25a^{*}b^{***}c^{ns}$
6	Group VI	$15.74\pm0.54a^{***}b^{***}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

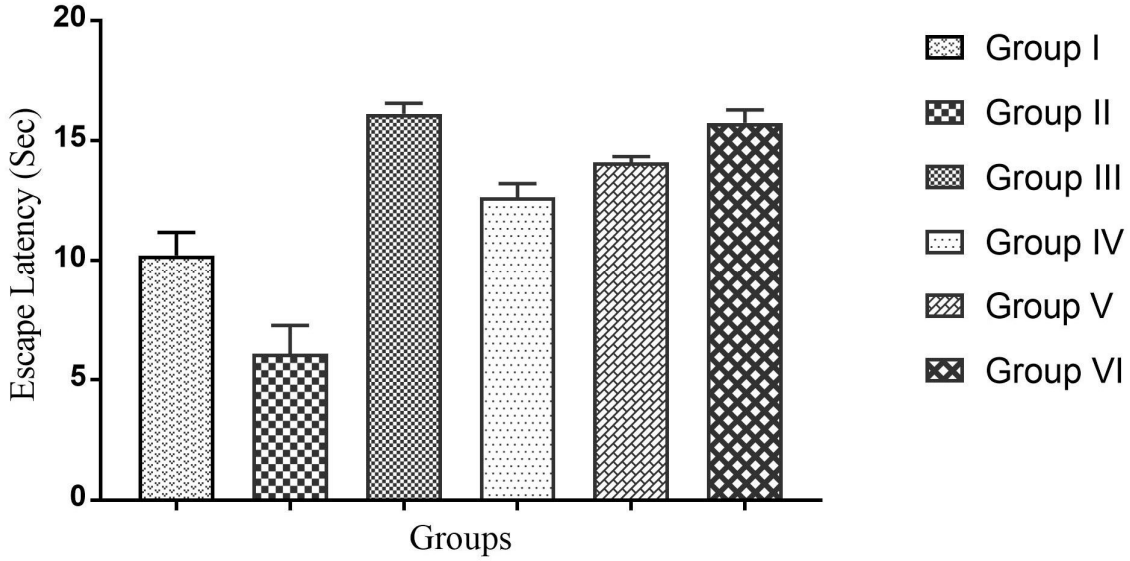


Figure 9

EFFECT OF AECL ON Y MAZE

Table 8

S.no	Groups	% Spontaneous alteration in the arms
1	Group I	47.85±1.45
2	Group II	$31.97\pm2.54a^{***}$
3	Group III	$42.95\pm1.32a^{ns}b^{**}$
4	Group IV	$32.73\pm1.11a^{***}b^{ns}c^{**}$
5	Group V	$35.13\pm1.36a^{***}b^{ns}c^{*}$
6	Group VI	$39.56\pm1.78a^{*}b^{*}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

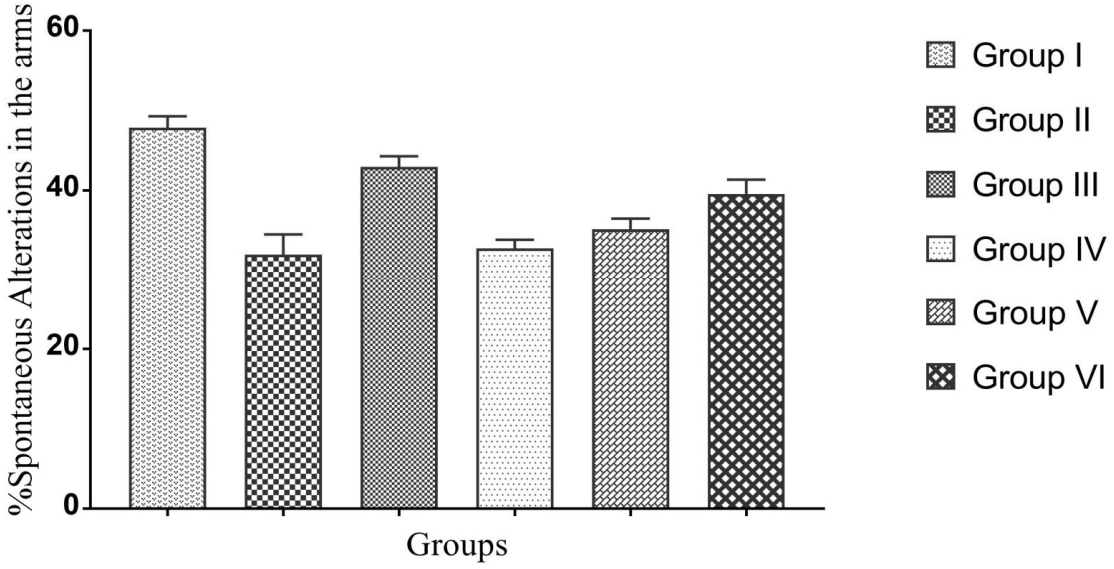


Figure 10

EFFECT OF AECL ON ACETLCHOLINESTERASE

Table 9

S.no	Groups	AChE µmoles/min/mg tissue
1	Group I	17.53±1.45
2	Group II	$32.40\pm1.26a^{***}$
3	Group III	$18.31\pm0.45a^{ns}b^{***}$
4	Group IV	$28.46\pm1.83a^{***}b^{ns}c^{***}$
5	Group V	$23.18\pm1.18a^{*}b^{***}c^{ns}$
6	Group VI	$24.46\pm0.68a^{**}b^{**}c^{*}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

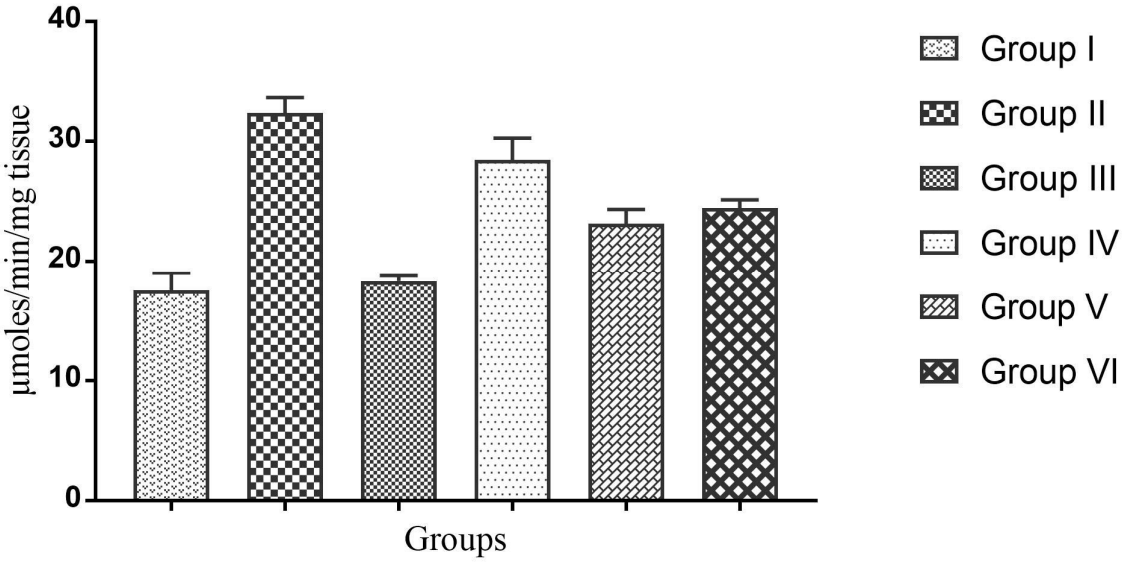


Figure 11

EFFECT OF AECL ON LIPID PEROXIDATION

Table 10

S.no	Groups	LPO nmol MDA/min/mg protein
1	Group I	1.18±0.07
2	Group II	$3.12\pm0.26a^{***}$
3	Group III	$1.59\pm0.36a^{ns}b^{**}$
4	Group IV	$1.65\pm0.11a^{ns}b^{**}c^{ns}$
5	Group V	$1.21\pm0.28a^{ns}b^{***}c^{ns}$
6	Group VI	$1.86\pm0.34a^{ns}b^{*}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

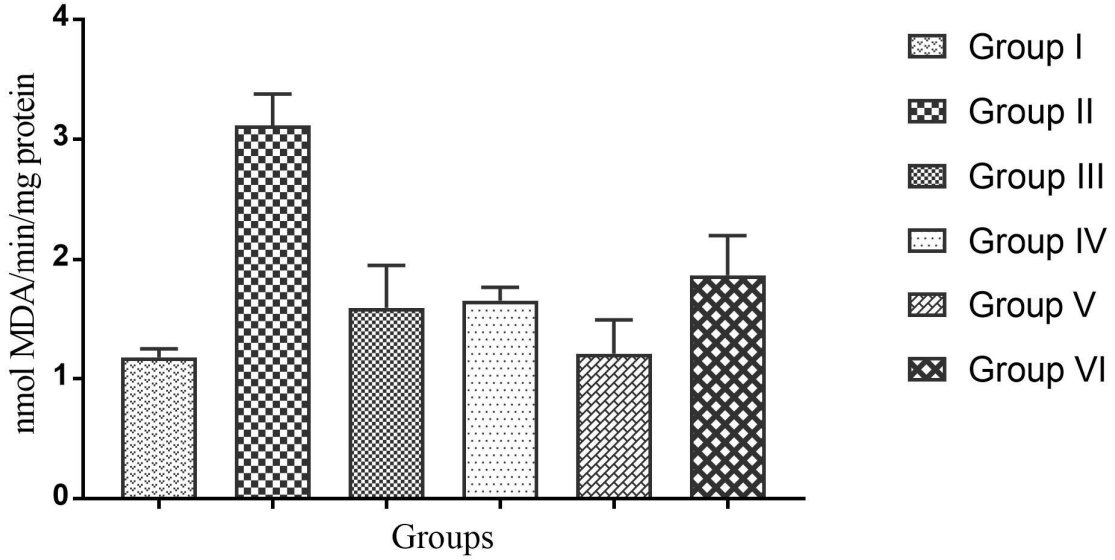


Figure 12

EFFECT OF AECL ON SUPEROXIDE DISMUTASE

Table 11

S.no	Groups	SOD Unit/min/mg of protein
1	Group I	95.51±0.34
2	Group II	$90.52\pm0.62a^{***}$
3	Group III	$93.36\pm0.45a^{ns}b^{*}$
4	Group IV	$94.42\pm0.83a^{ns}b^{***}c^{ns}$
5	Group V	$92.20\pm0.58a^{**}b^{ns}c^{ns}$
6	Group VI	$93.18\pm0.43a^{ns}b^{*}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

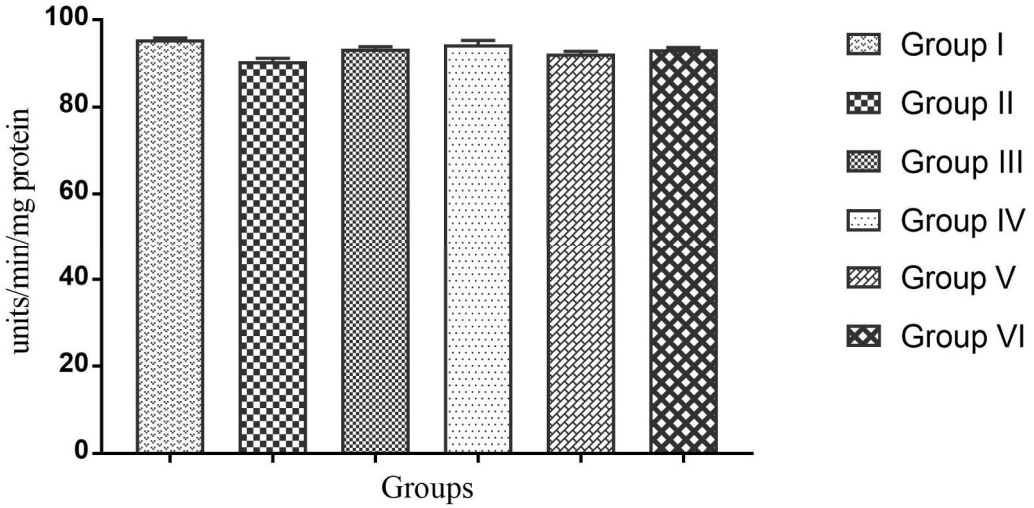


Figure 13

EFFECT OF AECL ON GLUTATHIONE PEROXIDASE

Table 12

S.no	Groups	GPx Units/mg protein
1	Group I	0.13±0.05
2	Group II	$0.21\pm0.03a^{ns}$
3	Group III	$0.07\pm0.03a^{ns}b^{ns}$
4	Group IV	$0.15\pm0.04a^{ns}b^{ns}c^{ns}$
5	Group V	$0.14\pm0.08\ a^{ns}b^{ns}c^{ns}$
6	Group VI	$0.18\pm0.06\ a^{ns}b^{ns}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

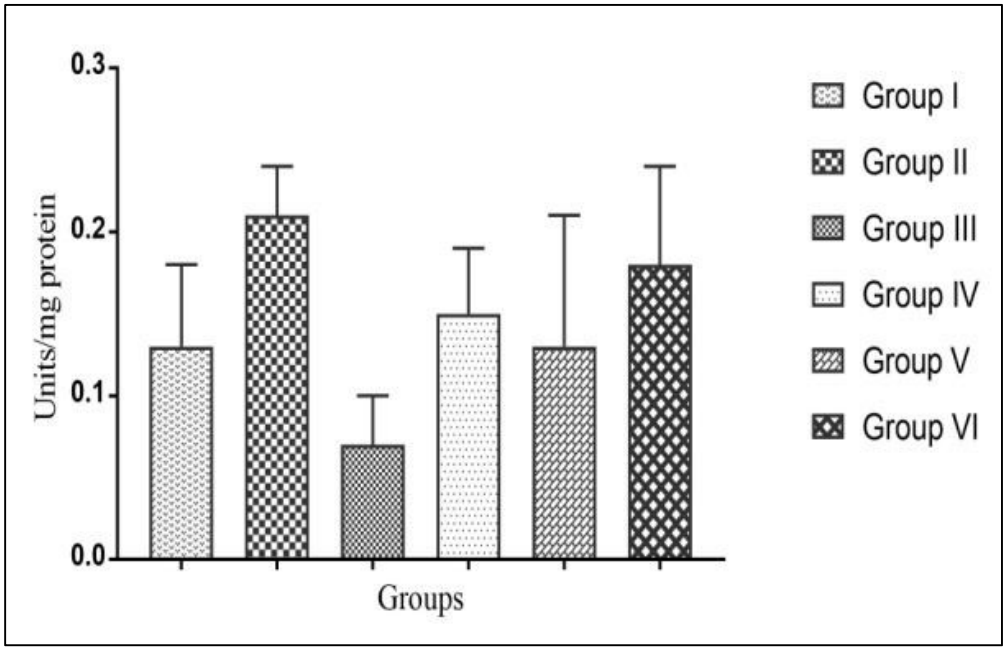


Figure 14

EFFECT OF AECL ON CATALASE

Table 13

S.no	Groups	CAT µmole of H2O2 consumed/min/mg protein
1	Group I	3.26±0.26
2	Group II	$4.15\pm0.21a^{*}$
3	Group III	$5.12\pm0.11a^{***}b^{*}$
4	Group IV	$3.54\pm0.18a^{ns}b^{ns}c^{***}$
5	Group V	$4.54\pm0.20a^{**}b^{ns}c^{ns}$
6	Group VI	$4.76\pm0.19a^{***}b^{ns}c^{ns}$

All the values are expressed as mean±SEM. ***-P<0.001, **-P<0.01, *-P<0.05, ns-non significant;

Group I Vs Group II, III, IV, V and VI is considered as a

Group II Vs Group III, IV, V and VI is considered as b

Group III Vs Group IV, V and VI is considered as c (one-way ANOVA followed by Dunnett's test).

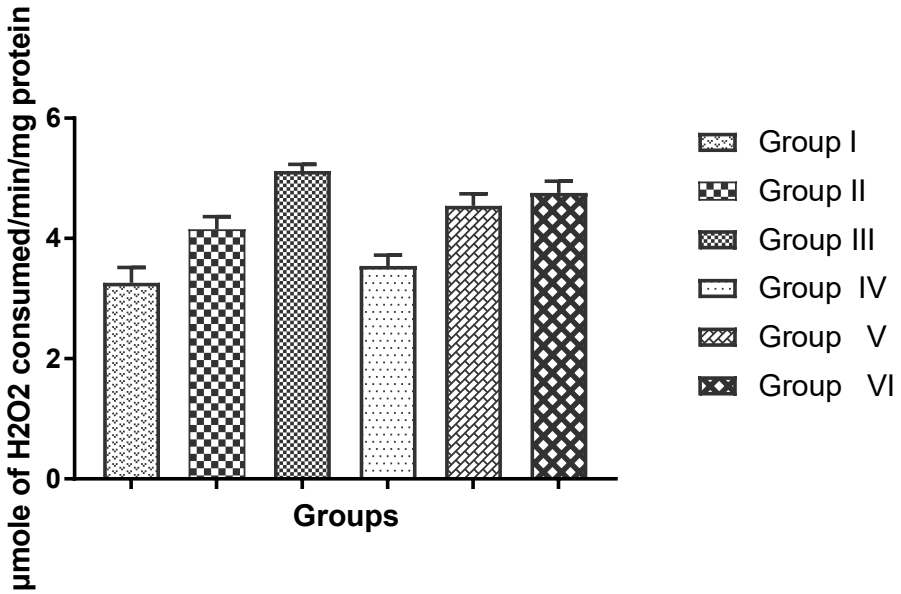
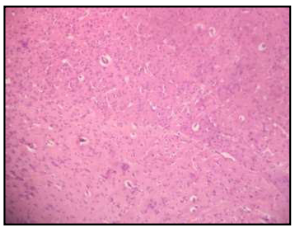


Figure 15

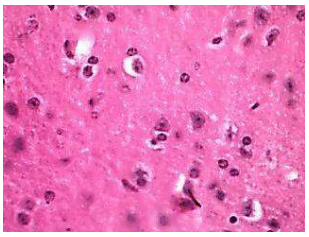
HISTOPATHOLOGY

Histopathology of brain

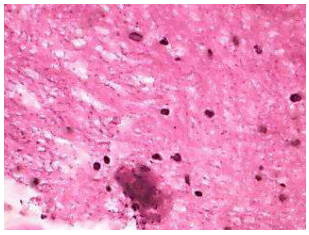


Group I

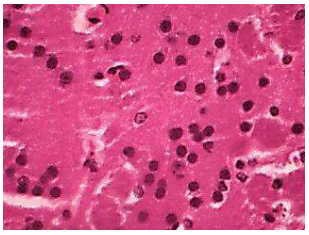
Group II



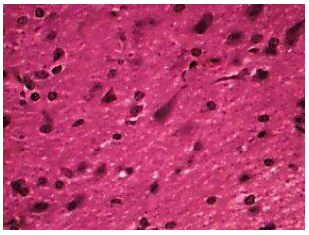
Group III



Group IV

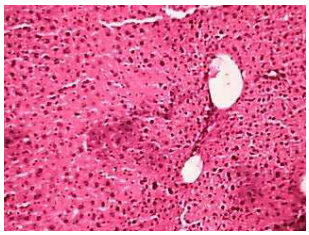


Group V

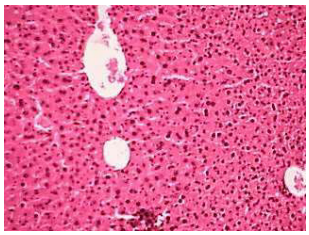


Group VI

Histopathology of Liver

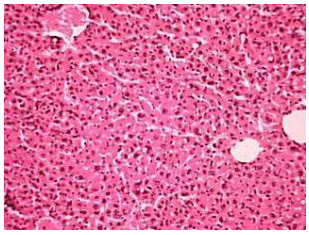


GroupI

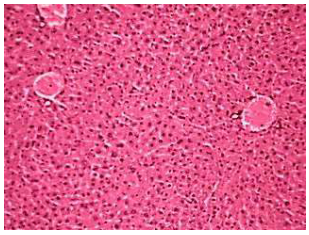


Group II

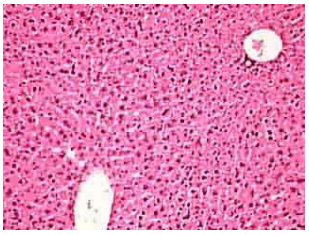
Group III



Group IV

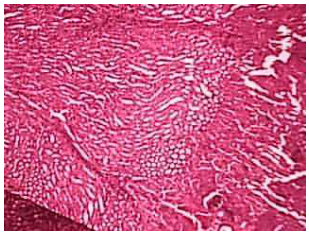


Group V

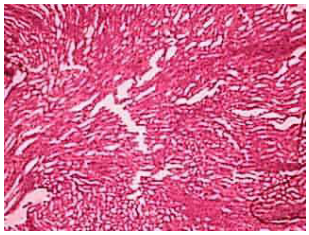


Group VI

Histopathology of Kidney



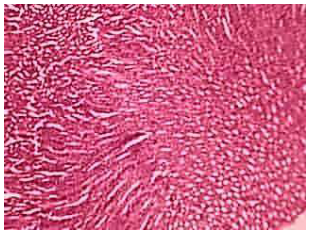
Group I



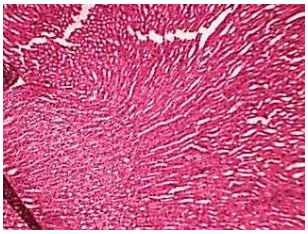
Group II

Group III

Group IV



Group V



Group VI

8. RESULTS

8.1. Preliminary Phytochemical analysis of Aqueous Extract of *Citrullus lanatus*(Thunb.) (AECL)

The result of preliminary phytochemical analysis of aqueous Extract of *Citrullus lanatus*(Thunb.). showed presence of various phytochemical constituents such as Phenols, Flavonoids, tannins, steroids, alkaloids, glycoside, saponins and with absence of Carbohydrates, terpenes, sterol, protein, gums and mucilage. (Table 4)

8.2. Behavioural assessment

8.2.1. Effect of AECL on Passive Avoidance Test

There was a significant difference between groups in the retention test.

When compared to Group I, Group II($P<0.001$) significantly decreased, Group IV($P<0.001$) and V($P<0.01$) significant, Group III and VI non significant and increased in the retention test.

When compared to Group II, Group III, V and VI significant ($P < 0.001$), Group IV non significant and increased latency during retention test.

When compared to Group III, Group IV significant ($P<0.01$) Group V and VI non significant and decreased latency in retention test.

Results were shown in figure 7, table 5

8.2.2. Effect of AECL on Elevated Plus maze

Transfer Latency (TL) on retention of information or memory.

When compared to Group I, Group II, III and VI significantly increased ($P < 0.001$) Group V non significant and decreased, Group IV($P<0.01$) significantly increased TL.

When compared to Group II, Group III, IV, V and VI significantly decreased TL ($P < 0.001$)

When compared to Group III, Group IV and VI ($P<0.001$), Group V ($P<0.01$) significantly increased TL.

Results were shown in figure 8, table 6

8.2.3. Effect of AECL on Morris Water Maze Test

In the probe trial followed by last training session, Escape latency was calculated

When compared to Group I, Group II ($P<0.01$) significantly decreased, Group III and VI ($P < 0.01$), Group V($P<.0.05$) significant and Group IV non significant and increased EL.

When compared to Group II, Group III, IV, V and VI significantly increased EL ($P < 0.001$)

When compared to Group III, Group IV ($P<0.05$) significant, Group V and VI non significant decreased EL.

Results were shown in figure 9, table 7

8.2.4. Effect of AECL on Y maze

In the probe trial followed by last training session,

When compared to Group I, Group II, IV and V significant ($P < 0.001$), Group III non significant, Group VI significant ($P<0.05$) and decreased Spontaneous alternation.

When compared to Group II, Group III significant ($P<0.05$), Group IV and V non significant, Group VI significant ($P<0.05$) and increased Spontaneous alternation.

When compared to Group III, Group IV significant ($P<0.01$), Group V($P<0.05$) and Group VI non significant and decreased Spontaneous alternation.

Results were shown in figure 10, table 8

8.3. Estimation of Neurotransmitter

8.3.1. Effect of AECL on Acetylcholinesterase

When compared to Group I, Group II and IV ($P<0.001$), Group V ($P<0.05$) and Group VI ($P<0.01$) significant and Group III non significant with increased level of AChE.

When compared to Group II, Group III and V ($P<0.001$), Group VI ($P<0.05$) significant and Group IV non significant with decreased level of AChE.

When compared to Group III, Group IV ($P<0.001$), Group VI ($P<0.05$) significant and Group V non significant with increased level of AChE,

Results were shown in figure 11, table 9

8.4. Estimation of Antioxidant

8.4.1. Effect of AECL on Lipid Peroxidation

When compared to Group I, Group II ($P<0.001$) significant, Group III, IV, V and VI non significantly increased the level of LPO.

When compared to Group II, Group III and IV ($P<0.01$), Group V ($P<0.001$) and Group VI ($P<0.05$) significantly decreased the level of LPO.

When compared to Group III, Group IV and VI non significantly increased and Group V non significantly decreased the level of LPO.

Results were shown in figure 12, table 10

8.4.2. Effect of AECL on Superoxide Dismutase Level

When compared to Group I, Group II ($P < 0.001$), Group V ($P<0.01$) significant, Group II, IV and VI non significant and decreased the level of SOD.

When compared to Group II, Group III and VI ($P<0.05$) and IV ($P<0.001$) significant, Group V non significant and increased the level of SOD.

When compared to Group III, Group IV non significant and increased, Group V and VI non significant and decreased the level of SOD.

Results were shown in figure 13, table 11

8.4.3. Effect of AECL on Glutathione Peroxidation

When compared to Group I, Group II, IV, V and VI non significantly increased and Group III non significantly decreased the level of GPx.

When compared to Group II, Group III, IV, V and VI non significantly decreased the level of GPx .

When compared to Group III, Group IV, V and VI non significantly increased the level of GPx. Results were shown in figure 14, table 12

8.4.4. Effect of AECL on Catalase

When compared to Group I, Group II ($P<0.05$) and Group III and VI ($P<0.001$), Group IV non significant and Group V ($P<0.01$) and increased the level of CAT.

When compared to Group II, Group III ($P<0.05$), Group V and VI non significantly increased and Group IV non significantly decreased the level of CAT.

When compared to Group III, Group IV ($P<0.001$) significant, Group V and VI non significant and decreased the level of CAT.

Results were shown in figure 15, table 13

8.5. HISTOPATHOLOGY

Table 14

Brain

GROUP	REPORT
Group I	Haematoxylin and Eosin stained section shows the normal brain tissue depicted intact cell architecture with normal amount of neurotransmitters.
Group II	Haematoxylin and Eosin stained section shows there is less neuron density.
Group III	Haematoxylin and eosin stained section of the brain tissue showed no significant alterations observed in this group and tissues showed a normal picture or brain cells, less proliferation and more neuronal density at hippocampal region
Group IV	Haematoxylin and Eosin stained section of the brain tissue showed no pathological damages and cellular architecture are intact with more neuronal density
Group V	Haematoxylin and Eosin stained section of the brain tissue showed no pathological damages and cellular architecture are intact with more neuronal density
Group VI	Haematoxylin and Eosin stained section of the brain tissue showed increased neuron density

HISTOPATHOLOGY

Liver

Table 15

GROUP	REPORT
Group I	Haematoxylin and Eosin stained section shows the normal liver tissue, no significant change was observed
Group II	Haematoxylin and Eosin stained section showed congestion of glomerulus and interstitial fibrosis
Group III	Haematoxylin and eosin stained section showed no significant alterations.
Group IV	Haematoxylin and Eosin stained section showed showed decreased amount of connective tissue in the glomerulus and interstitial fibrosis.
Group V	Haematoxylin and Eosin stained section showed showed reduced amount of connective tissue in the glomerulus and interstitial fibrosis
Group VI	Haematoxylin and Eosin stained section showed showed decreased amount of connective tissue in the glomerulus and interstitial fibrosis

HISTOPATHOLOGY

Kidney

Table 16

GROUP	REPORT
Group I	Haematoxylin and Eosin stained section shows the normal kidney tissue, hepatocyte showed normal lobular architecture
Group II	Haematoxylin and Eosin stained section showed hepatocyte with microcellular fatty changes with inflammatory cell
Group III	Haematoxylin and eosin stained section showed no significant alterations observed
Group IV	Haematoxylin and Eosin stained section showed minimal microcellular fatty change
Group V	Haematoxylin and Eosin stained section showed minimal microcellular fatty change
Group VI	Haematoxylin and Eosin stained section showed minimal microcellular fatty change

10. DISCUSSION

Choline is a primary component acetylcholine, an excitatory neurotransmitter in the nervous system that acts to enhance the message across neurons. The cholinergic system has been implicated in learning and memory. Loss of cholinergic receptor in the cortex correlates with the age related memory loss and cognitive deficits. Degeneration of cholinergic system is the central aspect of Alzheimer's disease. Loss of cholinergic cells is likely to underline the learning and memory deficits.[96]

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities[97]Which mostly affects the elderly population. The pathophysiology of AD is complex including defective beta-amyloid (Aβ) protein metabolism, abnormalities of glutaminergic, cholinergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the potential involvement of inflammatory and oxidative pathways[98] Hence, the present study focuses on exploration of the memory enhancing activity of the aqueous extract of *citrullus lanatus* (Thunb.) in scopolamine induced amnesia mice model.

Watermelon juice is a rich source of phenolics, α tocopherol, carotenoids such as beta carotene and lycopoene, and vitamin C[99,100] The beneficial effects of vitamin C are attributed mainly to its antioxidant properties.[101] Watermelon juice is an excellent source of lycopene, having about 40% higher lycopene content than raw tomatoes [102,103]. Studies have attributed the antioxidant properties of water melon juice to its high lycopene content.[104,82] There is a compelling evidence for the antioxidant role of lycopene in animal models of toxicant induced toxicities. Lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system[105].

Piracetam, the first representation of a class of Nootropic agents, has been shown to improve memory deficits in individuals. Repeated injection of piracetam had improved learning abilities and memory capacities of laboratory animals[49]. Scopolamine, an anti-muscarinic agent, competitively antagonizes the effect of acetylcholine on the muscarinic receptors by occupying postsynaptic receptor sites with high affinity and increases AChE activity in the cortex and hippocampus. Scopolamine diminish cerebral blood flow due to cholinergic hypofunction. Scopolamine additionally triggers ROS, inducing free radical injury and an increase in a scopolamine-treated group brain MDA levels and deterioration in antioxidant status. Scopolamine induces neuro-inflammation by promoting high level of oxidative stress and pro inflammatory cytokines in the hippocampus. Scopolamine is proven to increase levels of APP and Tau. Administration of

scopolamine led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration.[48]

The present study evaluates, Scopolamine-induced cognitive dysfunction is extensively used to probe potential therapeutic agents attenuating cognitive deficits and the effect of AECL 25%, 50% and 100% in scopolamine-induced learning and memory impairment in mice. Screening methods such as the Morris water maze, elevated plus maze, passive avoidance paradigm and Y maze were performed to screen the effect of drugs. Furthermore, using mice brain homogenate AchE level, brain oxidative markers, histopathological studies were performed.

Passive avoidance task is fear-aggrevated test used to evaluate learning and memory in rodent models of CNS disorder [106] A method for evaluating passive avoidance- and escape-learning responses simultaneously has been developed for the study of learning and memory in mice. Prolongation of the step-down latency and shortening of the escape latency in the retention test depended on the strength of the voltage of the electric shocks delivered during the training test. Therefore, the step-down latency and escape latency may be good parameters of learning and memory performance and significant.[107]AECL increased SDL induced by i.p. treatment of scopolamine in the retention trail. This suggests that the animal has the retention of memory of the shock once entered in the shock-free zone.

An elevated plus-maze consisting of two open and two enclosed arms was employed for an evaluation of memory in mice. The results suggested that transfer latency may be one of the parameters of learning and memory Mice in the plus-maze escaped from the open arm to the enclosed arm because mice apparently dislike open and high spaces. The time it took for the mice to move from the open arm to the enclosed arm (transfer latency) was recorded[108]. In elevated plus maze, AECL decreases the transfer latency during probe trial indicated improvement of memory.

Morris water maze were employed as behavioral models for evaluation of learning and memory.[88] Morris water maze is generally accepted as an indicator of spatial learning and reference memory. It is a reliable and convenient method to assess hippocampal-dependent cognitive function in rodents[109].AECL reversed scopolamine-induced memory impairment in the MWM test by increasing the EL in the probe test. These results suggest that AECL attenuates long term and reference memory impairment induced by scopolamine through the rescue by antioxidant mechanisms and acetylcholine system.

Spontaneous alternation using a Y-maze is a test for habituation and spatial working memory[110] Spontaneous Alternation Tests are used to evaluate exploratory behaviour in mice. Brain areas involved in this test include hippocampus, septum, basal forebrain, and prefrontal cortex. AECL treated mice showed increase in spontaneous alterations than the scopolamine treated mice.

One of the most promising therapies to treat a cognitive deficit in AD is to increase the cholinergic activity and inhibition of AchE enzyme. In the brain, acetylcholine is produced in several locations including the basal forebrain. It may promote learning. Acetylcholine-producing cells in the basal forebrain are damaged in the early stages of Alzheimer's disease, which may contribute to the memory impairments which are an early symptom of the disease[111] AECL decreased the AChE level in brain compared to the scopolamine treated mice.AECL acts by diminishing the AChE, shows improvement in scopolamine induced amnesia in mice.

Molecular and cellular factors that contribute to the selective vulnerability of neurons to oxidative stress. ROS/RNS can serve as signaling molecules while they cause damages to bio-molecules at increased levels.

Lipid peroxidation causes cell membrane destruction and cell damage. The presence of a high concentration of oxidisable fatty acids and iron in liver significantly contributes to ROS production. Furthermore, the abundance of polyunsaturated fatty acids (PUFAs) and redox active transition metal ions in the brain in addition to its high oxygen usage makes it highly susceptible to oxidative damage. Scopolamine significantly elevated the malondialdehyde (MDA) levels in the brain indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. AECL treatment significantly reversed these alterations causing a significant decrease in MDA levels suggesting its protective effects against scopolamine-induced oxidative damage.

Suproxide Dismutase, an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage[112].AECL treated significantly increased level of SOD than scopolamine treated mice.

Glutathione preroxidase (GPX) is another enzymic anti-oxidant that acts as a defense against oxidative stress. There was no significant effect of GPx activity observed in our study after scopolamine treatment.

Catalase oxidation reaction occur in the presence of a hydrogen peroxide (H_2O_2) to form acetaldehyde. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). AECL treated mice showed increase in catalase than the scopolamine treated animals

Histopathological study of mice proved no pathological damages and cellular architecture are intact with more neuronal density in brain. In liver, reduced amount of connective tissue in the glomerulus and interstitial fibrosis and minimal microcellular fatty changes in kidney.Thus, Animal pre-treated with AECL protected the organs by reversing the damage induced by scopolamine.

The protective effect of *Citrullus lanatus*(Thunb.) may be due to lycopene content. Lycopene has been shown as a neuroprotective agent against Ab-induced neurotoxicity in primary cultured rat and it was suggested as a promising candidate for Alzheimer Disease treatment and proven evidence for the potential of lycopene in the management of scopolamine induced amnesia[113]

11.CONCLUSION

The result of the study shows, neuroprotective and improved cognitive effect of AECL in scopolamine induced animal model could be due to the synergistic effect of the phytohytoconstituents, further can be explored on the phytoconstituent present for valued treatment of AD

Anti oxidant study give the significant result.

Further, the significant increase of acetylcholinesterase level in the brain, liver, kidney of AECL shows further study is required to establish the mechanism of action of AECL

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INSTITUTE OF HERBAL SCIENCE
PLANT ANATOMY RESEARCH CENTRE

Prof.**P Jayaraman, Ph.D**
Director
Retd, Professor, Presidency College Chennai-5

AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /~~microscopic~~ examination of fresh /market sample, it is certified that the specimen given by Divya.N, Dept of Pharma-Cology C.L. Baid Metha college of Pharmacy. is identified as below:

Binomial: Citrullus lanatus (Thunb.) Matsumura & Nakai

Family: Cucurbitaceae

Synonym(s): Citrullus Vulgaris Schrader ex Eckl & Zeyher

Regional names: Tamil:- Dharbusini.; Eng: Watermelon.

Reg.No of the certificate: Parc/2017/3559

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Date: 09.11.2017

(Prof.P.JAYARAMAN)
Prof.P.Jayaraman,Ph.D.
Director,
Institute of Herbal Botany
PLANT ANATOMY RESEARCH CENTRE.
No.4-II Street,Sakthi Nagar,
West Tambaram,Chennai-45.
Ph.044-22263236, Cell:9994136959
E-mail:herbalparc@yahoo.com

#4,2nd Street, Sakthi Nagar,
West Tambaram, Chennai-600 045
Ph:044-22263236,+919444385098
Email- herbalparc@yahoo.com